

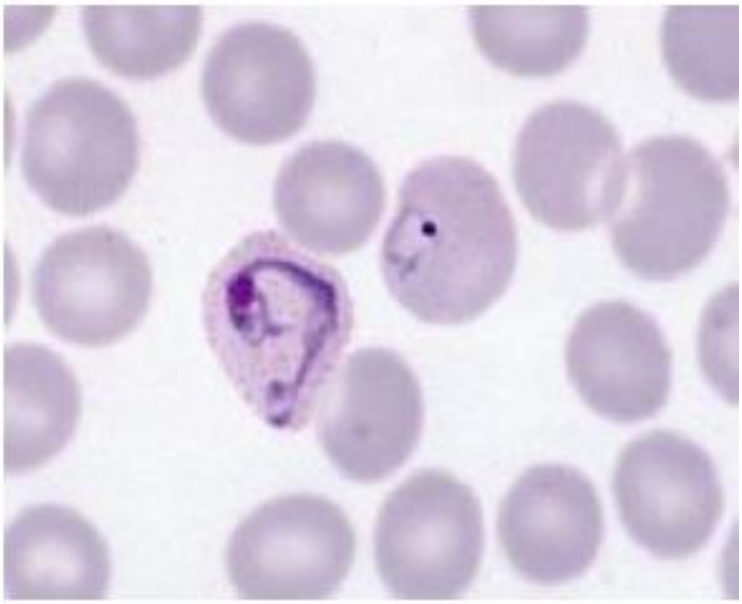
Bench Aid Handbook for the Microscopic Diagnosis of Malaria

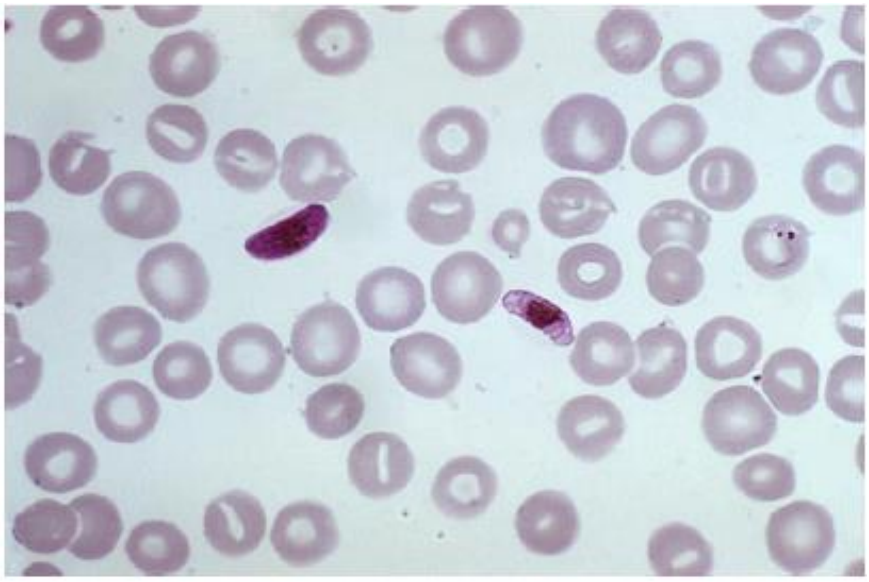
Compiled by Robin Wales

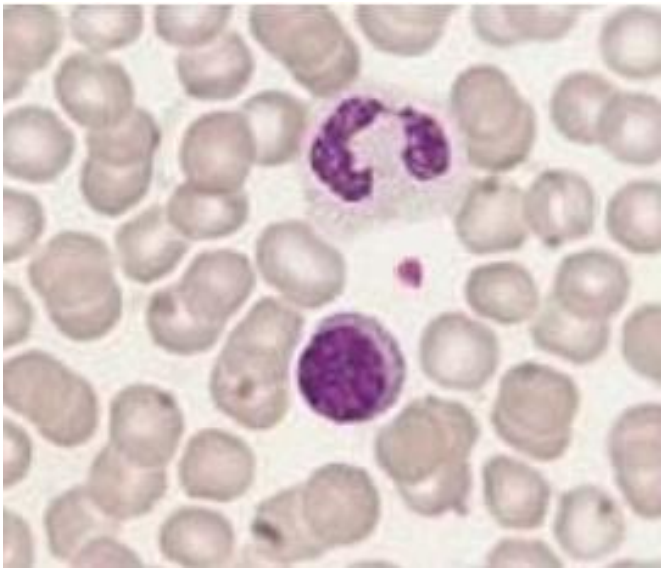
With grateful acknowledgement to:

Royal Perth Hospital Malaria Resource,
Copyright State of Western Australia.
Shoklo Malaria Research Unit.
CDC Public Image Library.
World Health Organisation.
Tropical Health Technology and Professor Balbir Singh for P knowlesi information.

All pictures are taken using 100 × oil immersion objective in Geimsa stained thick and thin blood films.







Normal blood film. Red and white blood cells and platelet.

No malarial parasites.

Microscopic examination of blood films

It is important that blood films for microscopy are well made and properly stained. Thin films must be spread evenly so that individual RBCs can easily be seen and examined in the thinner end of the film. Methanol used for thin film fixation must be pure and contain no water. The slides must be kept dry and fixed as soon as possible. Any contact with water will destroy the thin film.

Thick films can be unusable if the RBCs become fixed and are not washed away during staining. This may happen if slides are stored for too long or put in hot places. It can also happen if methanol, or the vapour from the methanol container, comes in contact with the thick film when the thin film is being fixed.

Thick films are used for diagnosis of malaria. It is only necessary to examine the thin film in order to identify the species if the thick film was positive, or to report the percentage of RBCs containing Pf parasites. This is sometimes done during treatment to check the result of medication.

Thick films should first be examined using the 10x and 40x microscope objectives. We do this to select best stained parts of the slide where we will examine closer. The best part of the thick film has about 10 White blood cells per High Power Field (HPF). It is sometimes possible to identify other things at this stage, including Microfilaria. We may also see dark brown Malaria pigment in WBCs, this is an important finding even if no parasites are found because it is a clue that malaria parasites have been in the cells.

Raise the objectives above the slide and put a small drop of immersion oil on the selected area before examining with the 100× objective.

Examine the film using a pattern as below, moving from one field to the next systematically, continue for 100 good fields. Report result as positive or neagative for malaria. If positive record species, stages and numbers of parasites.



This diagram shows the correct way to view the thick and thin sections of a blood film.

If the thick film is unusable (fixed, washed off etc) but the thin film is good, examine 500 fields in the thin film. If this result is negative a fresh slide should be requested and the thick film reported from the new slide.

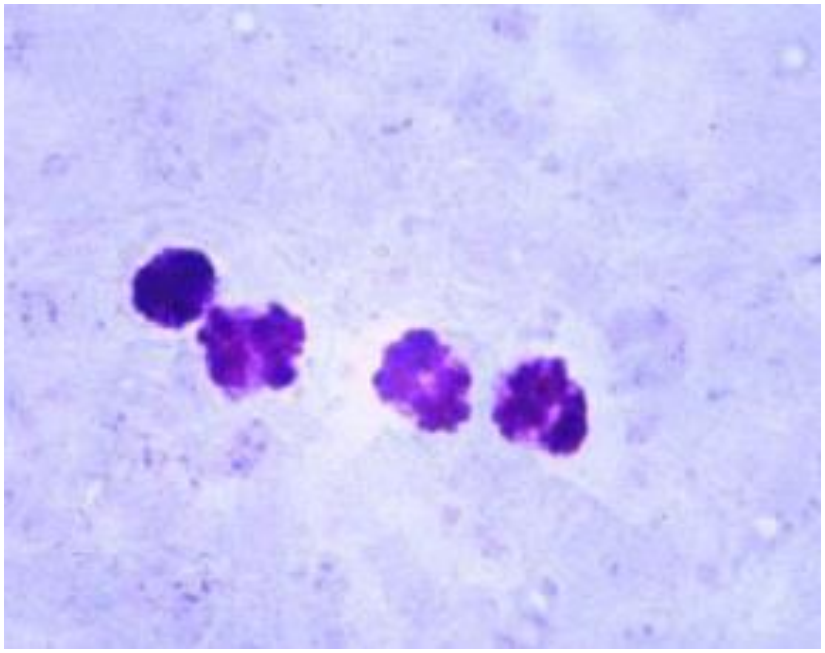
Most microscopes are supplied with 10× eyepieces. If possible, it is better to use 7× eyepieces as they give a clearer image with the 100× oil immersion objective. Never use eyepieces over 10×.

Reporting parasite numbers can be done in different ways. For routine reporting in thick films, with an average of 10 WBCs per HPF, use the following:

Number of Parasites	Report as	
Less than one parasite per HPF	Scanty or Rare	
1—2 parasite(s) per HPF	+	(1+)
3 –25 parasites per HPF	++	(2+)
26—60 parasites per HPF	+++	(3+)
Over 60 parasites per HPF	++++	(4+)

In thin films the number of Pf parasites can be expressed as a percentage of RBCs infected. This is done by counting the number of parasites in fields of view which contain a standard number of RBCs. E.g. calculate from the number of parasites in 5 fields with an average of 200 RBCs in each.

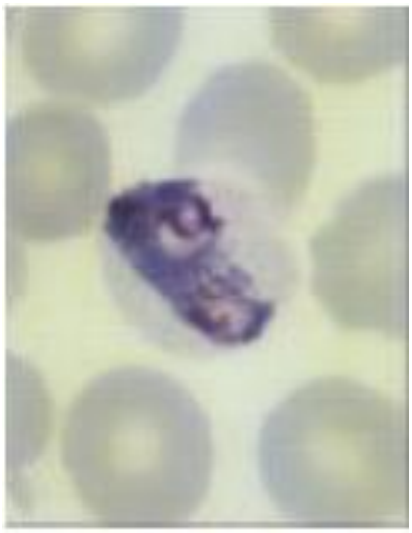
Sometimes parasite counts are made by comparing with the number of WBCs in thick films.



Thick film showing no malarial parasites. At least 100 fields must be examined.

Always use new or properly cleaned slides for making blood smears. Unclean slides, poor quality Geimsa stain, or water which is too acid or alkaline can result in unsatisfactory staining.

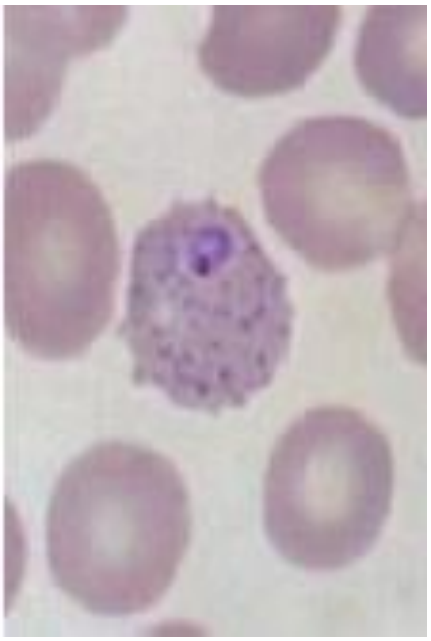
If the water used for diluting the Giemsa stain is slightly alkaline, the image will be too blue. If the water is slightly acid, the image will be too red. The pH of the water can be corrected using a pH 7.2 buffer made by adding buffer concentrate or buffer tablets to water.



Developing P.vivax trophozoite. Because the water used to dilute the Giemsa stain was alkaline, the slide is very blue. The Schüffners dots are not visible as a result.



P.vivax trophozoite. This is a well stained film. Note that the red cells and Pv trophozoite are pinker in this slide.



P.ovale trophozoite. Because it is well stained the James' dots are clearly visible.



P.ovale trophozoite. The water used to dilute the Giemsa stain was slightly acid, making the film appear too red. As a result the James' dots are only just visible.

Even using the same stain and staining method there will be variations in the colour and quality of staining in the slides you examine. You will notice these differences in stain quality and parasite appearance in the photos in these notes. It is important to learn to recognise parasites in differently stained slides.

It is important for you to be able to recognise things which can be mistaken for a malaria parasite. These things include stain crystals, platelets and debris from dirty slides.

There are five species of malaria that infect humans:

They are transmitted by female Anopheles Mosquitoes. Two of these, Plasmodium falciparum (Pf) and Plasmodium vivax (Pv) are the most common in Southeast Asia and together cause over 95% of cases of Malaria. Overall occurrence of each is about 50%, with Pf more frequent in tropical areas and Pv in more temperate places.

Pf is the most important and causes many deaths, especially in children. It also causes serious problems for pregnant women and their unborn and newborn babies. We need to identify this quickly by microscopy or RDT because special medicine must be given as soon as possible.

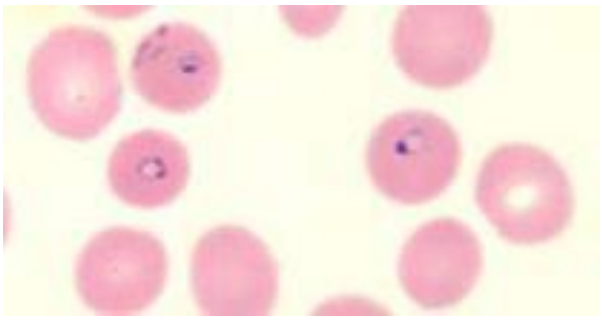
Pv is also an important cause of illness and loss of income. We can diagnose malaria and also see which species is causing it by malaria microscopy. The parasites are found inside red blood cells and differences in appearance of these for each species enable us to differentiate them.

The other two species, Plasmodium ovale - Po, and Plasmodium malariae - Pm, have both been found in Thailand and Burma, but are not common. Like Pv, they are less serious than Pf and respond well to treatment.

Plasmodium knowlesi is a malarial parasite in monkeys It is more and more commonly causing sever malarial illness in man. It is mainly found in Malaysia where large areas of forest have been cleared causing much higher risk of mosquitoes transferring the disease to humans.

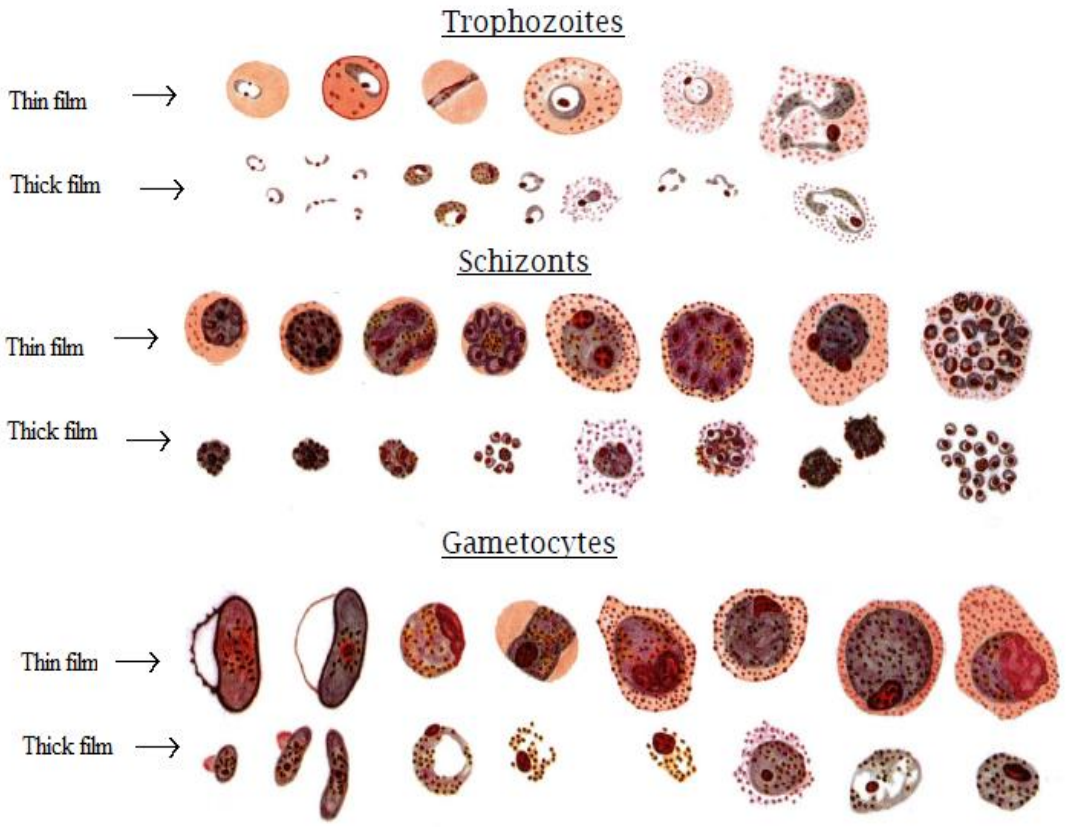
We will be using the scientific names given to the forms of malarial parasites at different stages of their growth and maturation inside red blood cells.



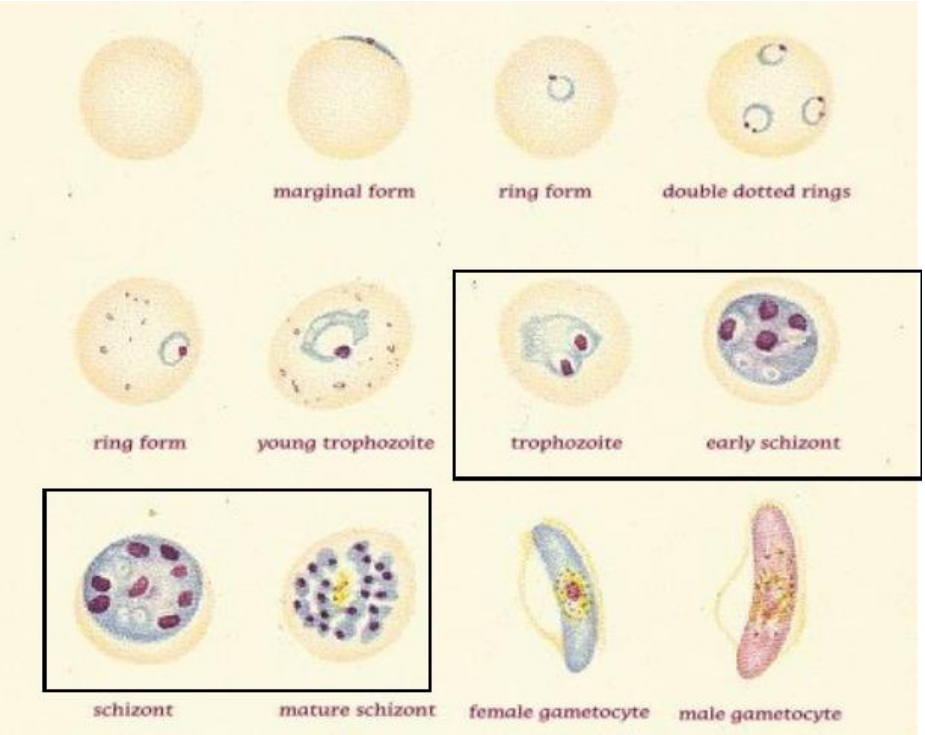


Tiny single cell merozoites, in left picture, from blood or liver schizonts enter red blood cells and become early ring form trophozoites, These are shown on the right with red nuclear dot and blue cytoplasm.

This diagram shows a comparison of all the Plasmodium species within their developmental stage. The thin film is shown on the top line and the thick film on the bottom line. The size and shape of parasites and infected red blood cells varies between species.

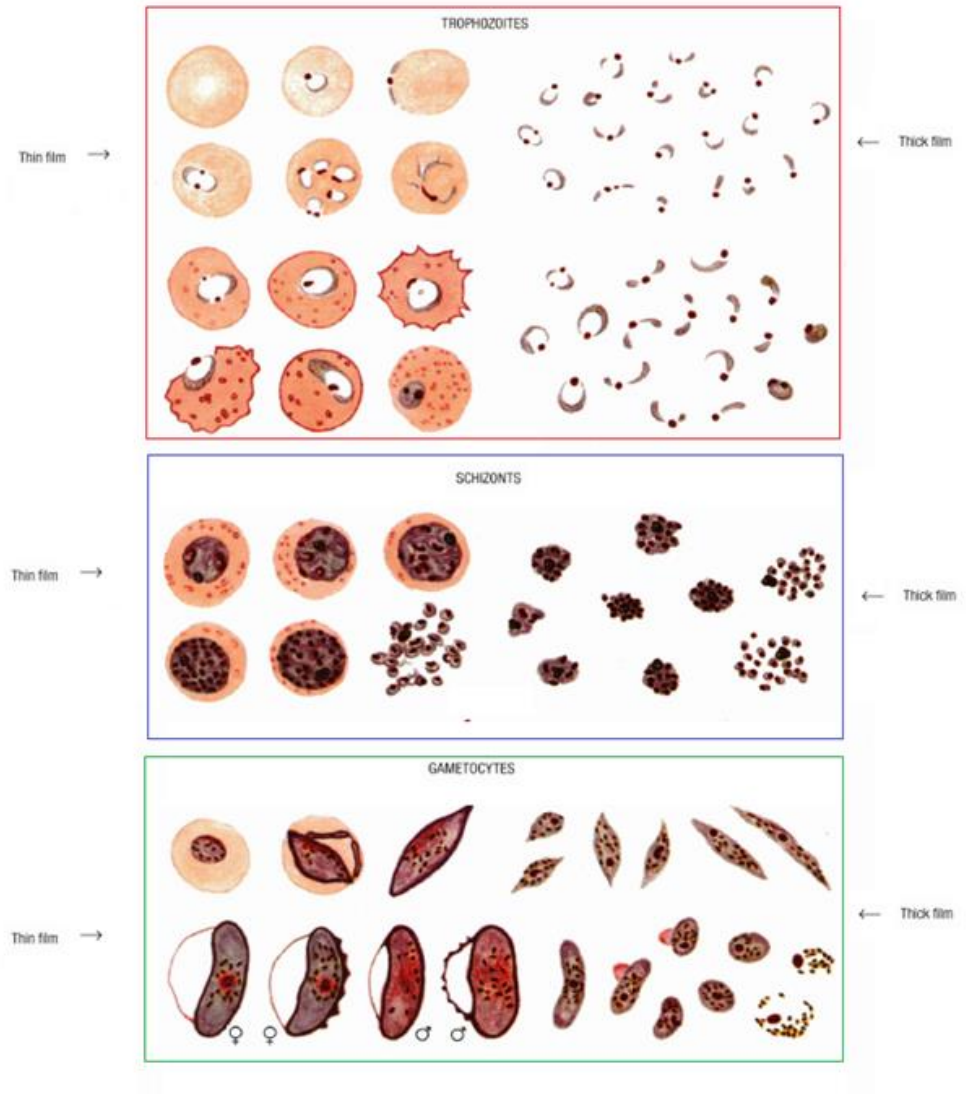


P.falciparum



This diagram shows the developmental stages of P. falciparum. We do not usually see the stages of P. falciparum parasites from young trophozoites to mature schizonts (shown in boxes) when we examine blood films. These stages occur in capillary blood vessels of deep inner organs and in the placenta of the pregnant woman.

Diagnosis of P. falciparum malaria is based on finding the ring form trophozoites and gametocytes characteristic of this species in thick and thin blood films. The typical shaped gametocytes enable us to make a certain diagnosis of Falciparum malaria.

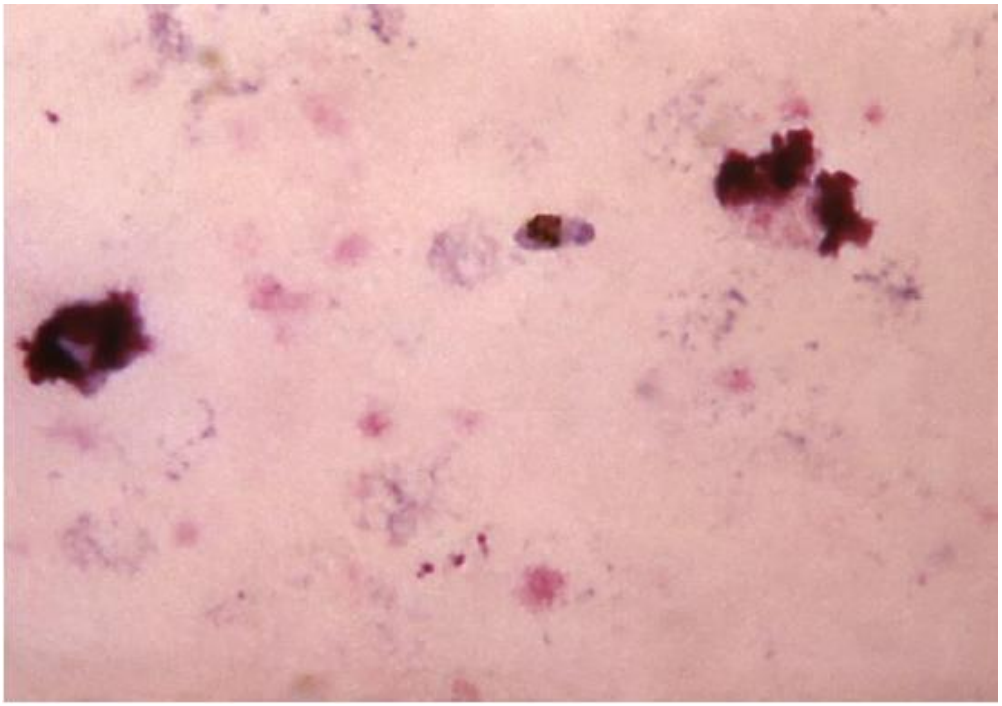


This diagram shows the appearance of the Pf parasite in the thin films on the left hand side, and thick films on the right. In this species the size of the red cells is not increased and they may have crenated (ragged) edges. Dots developing as trophozoites mature are sometimes seen in the thin films. These are Maurer's clefts.

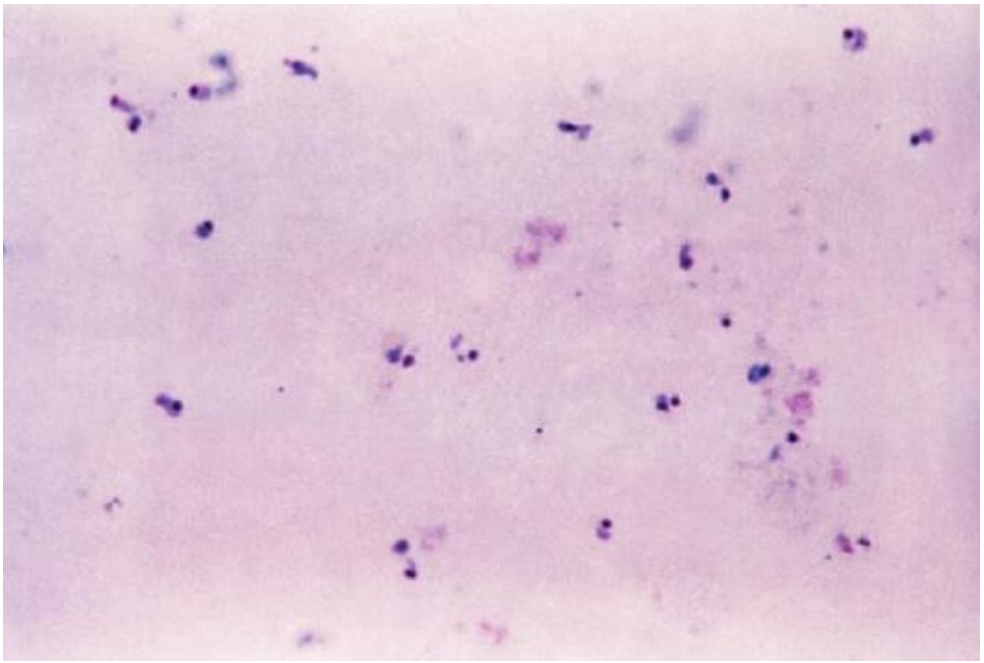
Identifying features of P.falciparum

- Usually only ring trophozoites and gametocytes are seen.
- The red cell size is not enlarged.
- The red cells are often crenated (jagged edges).
- Large numbers of small ring trophozoites are often present.
- Sometimes more than one parasite is seen in the red cells. Often two nuclei are seen. They may be on the edge of the red cells.
- No regular dots like the Schüffner's dots of Pv are present in early rings, but irregular dots called Maurer's clefts may be seen in maturing trophozoites.
- The gametocytes have a typical banana shape. This is diagnostic of Pf. Malaria pigment granules may be present, mainly around nucleus.

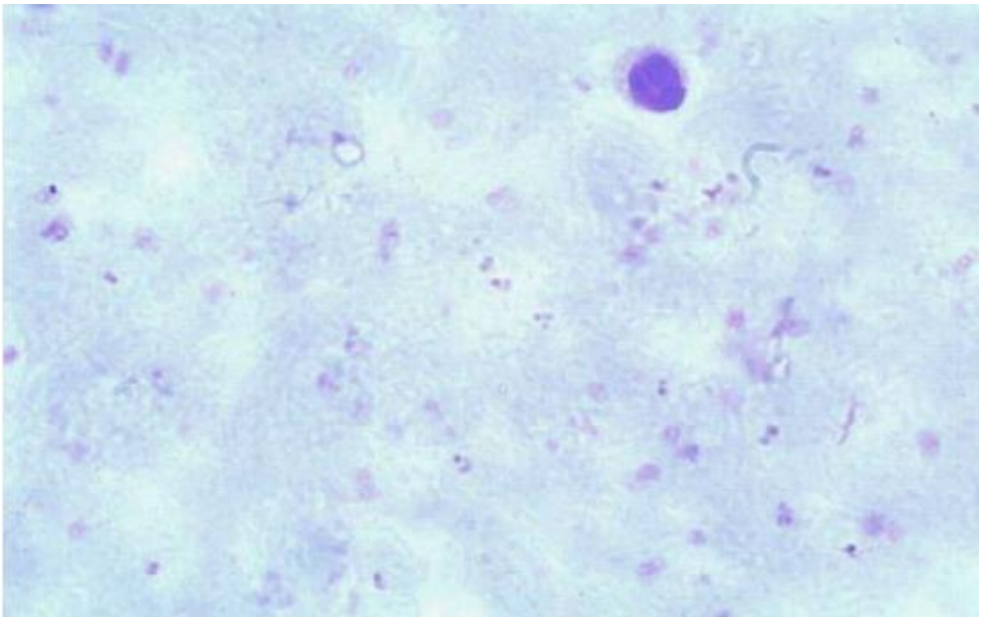
Thick films of P.falciparum



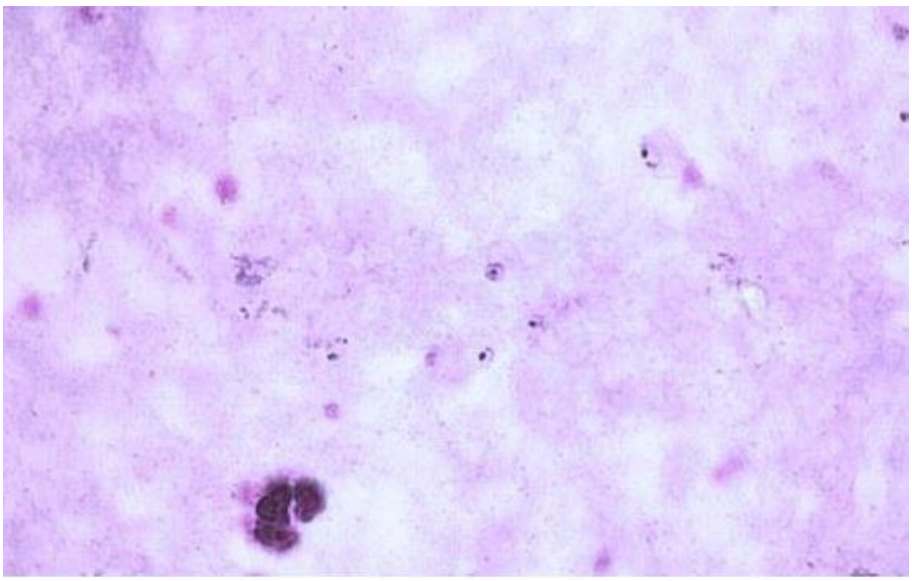
Thick film showing Pf ring trophozoites and gametocyte.



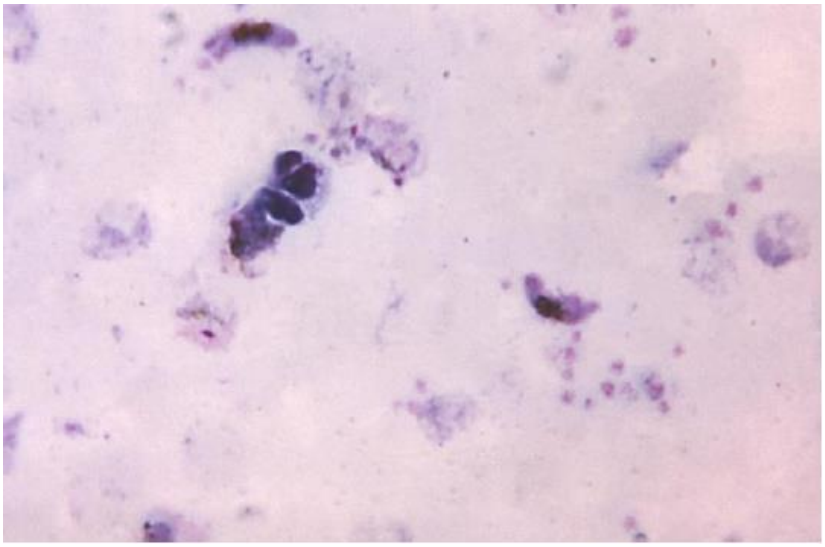
Thick film showing Pf growing trophozoites.



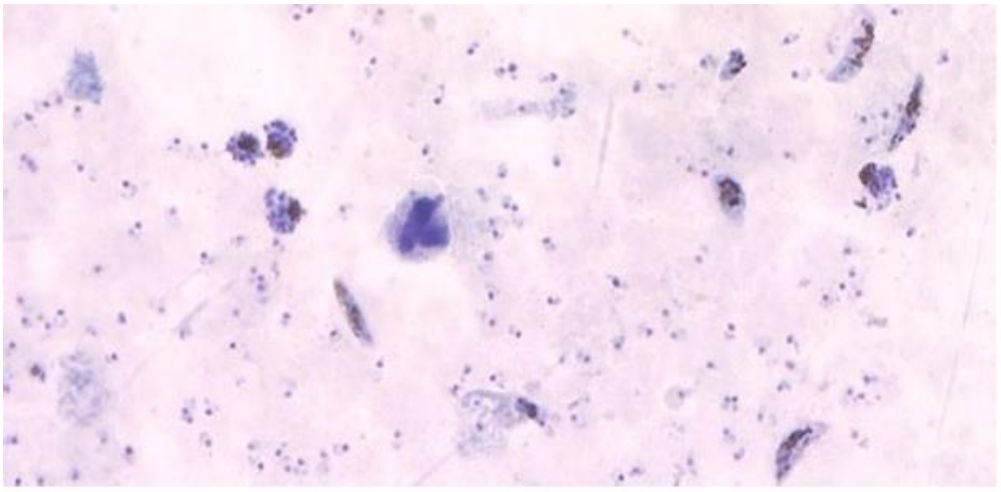
Pf ring trophozoites.



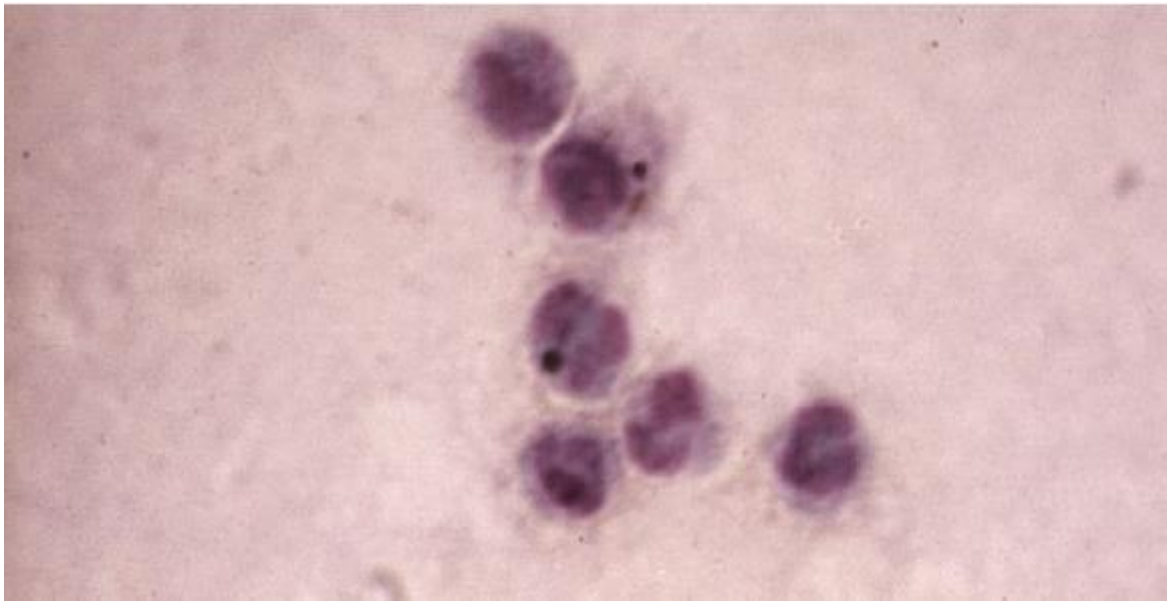
Pf ring trophozoites.
Notice the difference in staining qualities of this image and the previous one.



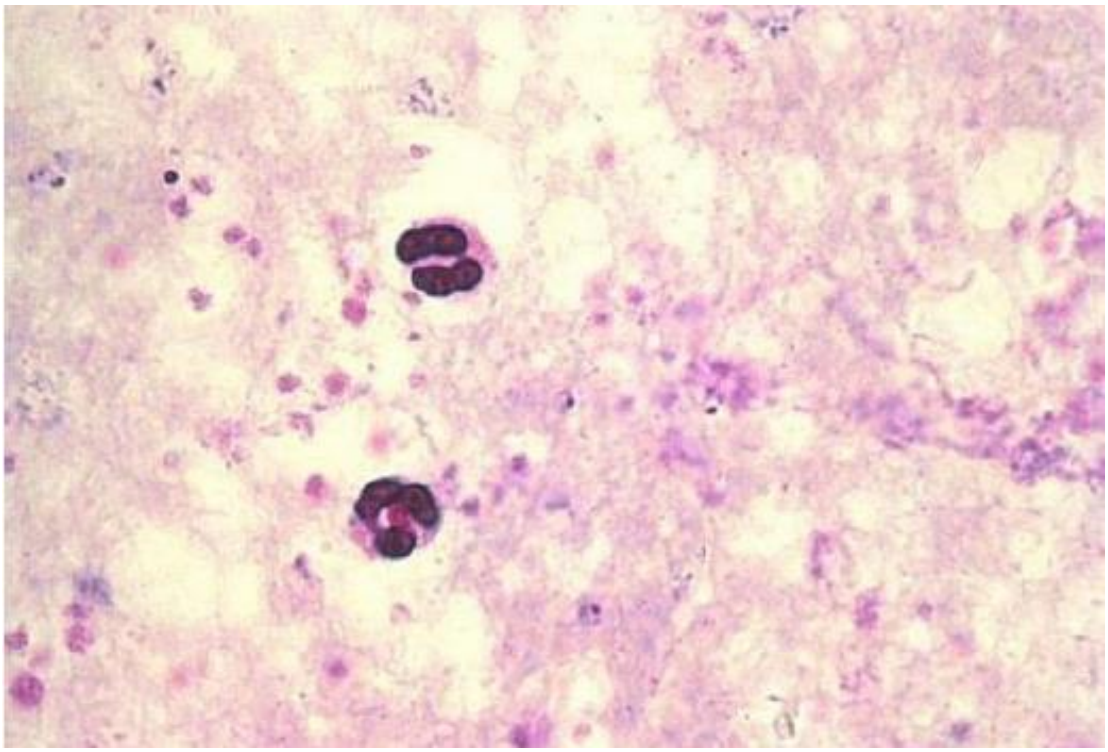
Mixed infection of Pf gametocytes and Pv trophozoites.



Many Pf rings, four distinct gametocytes, and schizonts. Schizonts are small and rarely seen in the circulating blood, except in very severe malaria. They contain two to four, and occasionally up to eight merozoites.



Malaria pigment in two white blood cells.

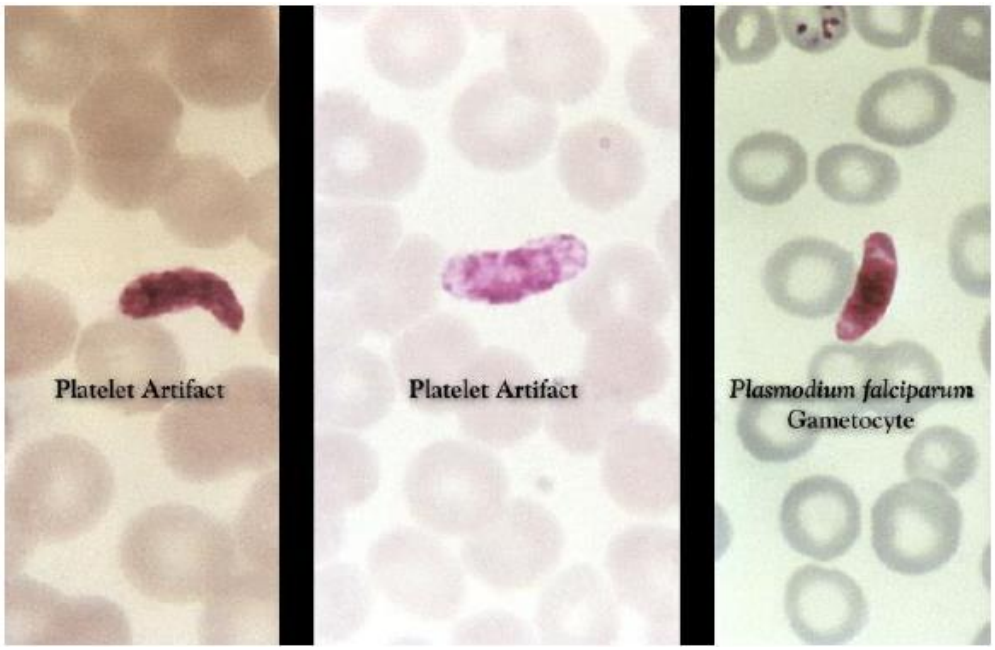


A few Pf rings and many platelets.

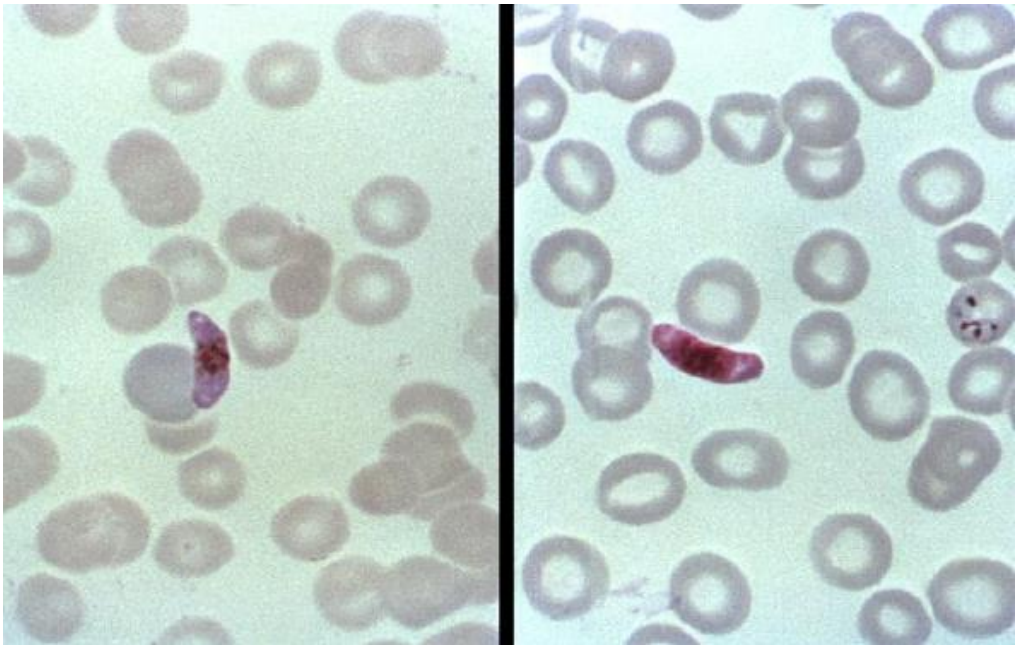
Thin films of P.falciparum



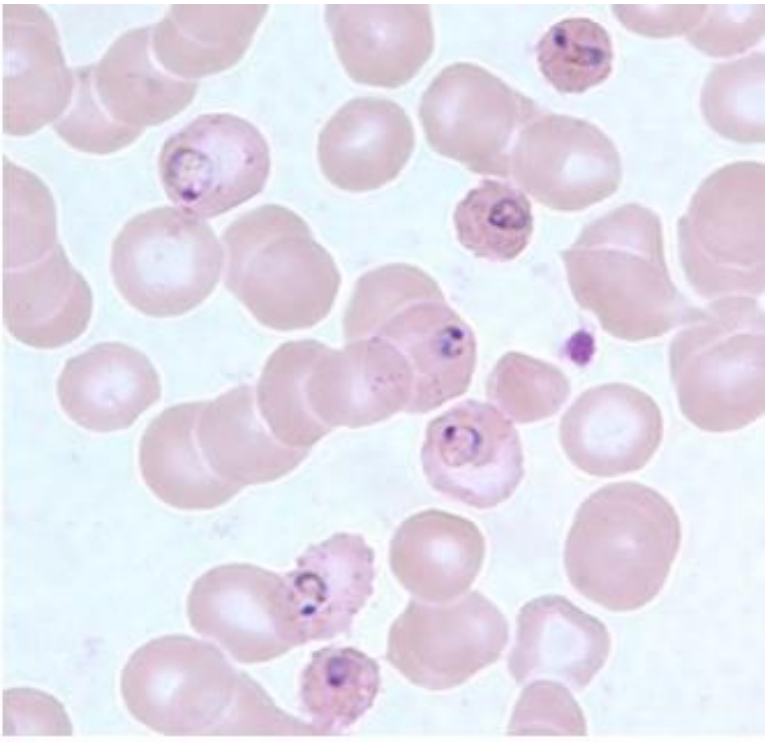
Malaria pigment in a WBC. This is an important finding and must always be reported.



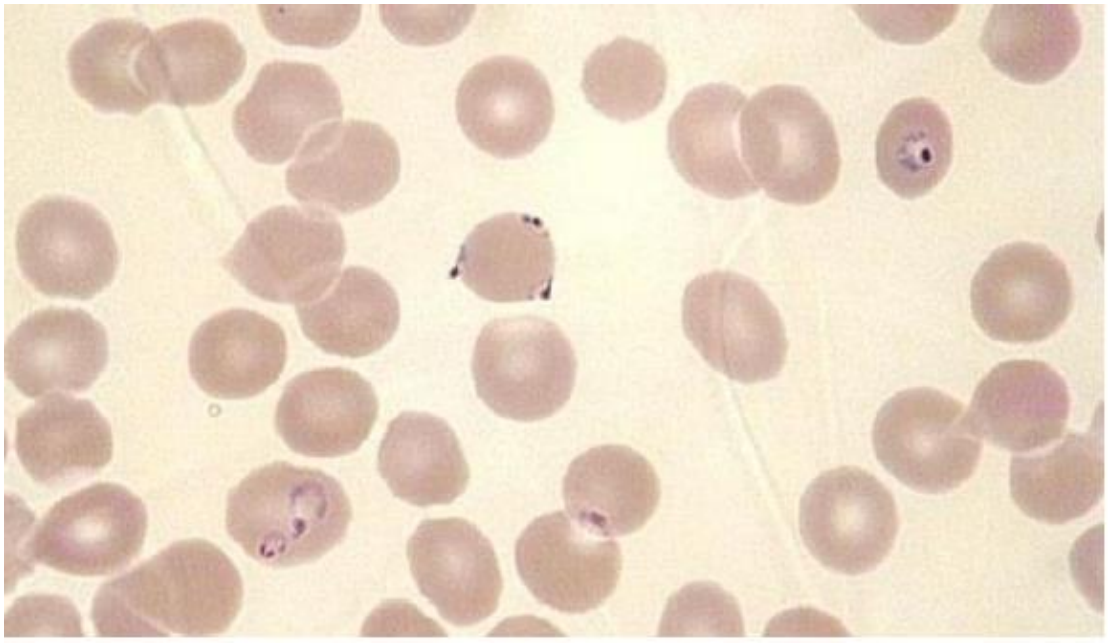
Thin films showing two platelet artefacts and a Pf gametocyte. It is important to be able to tell the difference between the two.



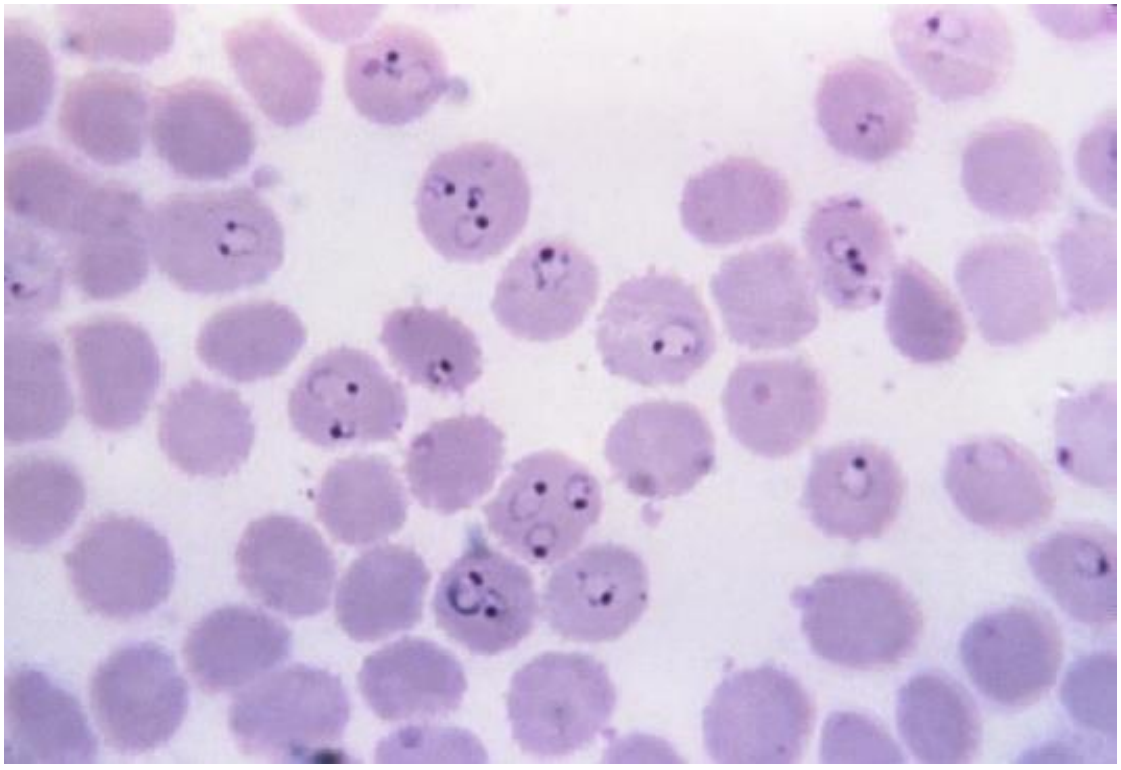
Thin films showing a female gametocyte of Pf on the left and male gametocyte of Pf on the right.



Pf trophozoites with Maurer’s clefts and crenated red cells.

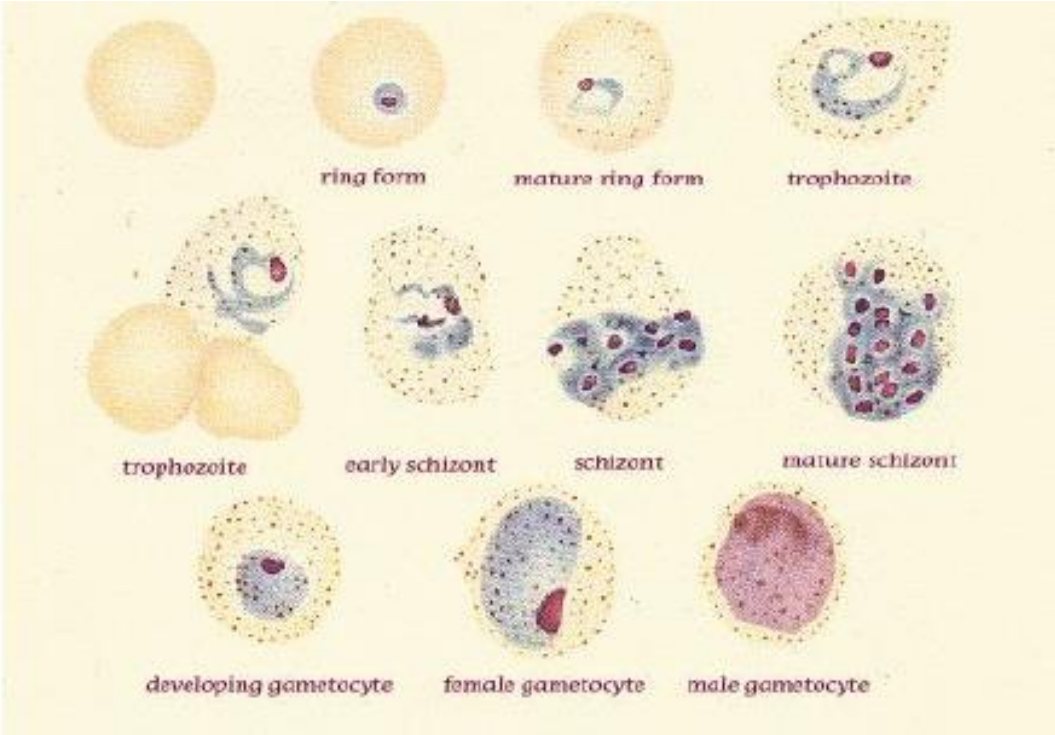


Pf rings with three on the wall of one red cell. This is called the accolé form.



Heavy Pf infection with multiple rings in RBCs, double dots and crenated RBCs.

P.vivax



The last diagram shows the developmental stages of P. vivax. All stages of trophozoites, schizonts, and gametocytes can be found in thick and thin blood films.

Identifying features of P.vivax

Red cell size increases greatly as parasites grow and mature. The RBC is often irregular in shape due to the amoeboid nature of the parasite.

Spreading cytoplasm becomes irregular and fragmented during growth and many Schüffners dots are seen in well stained films.

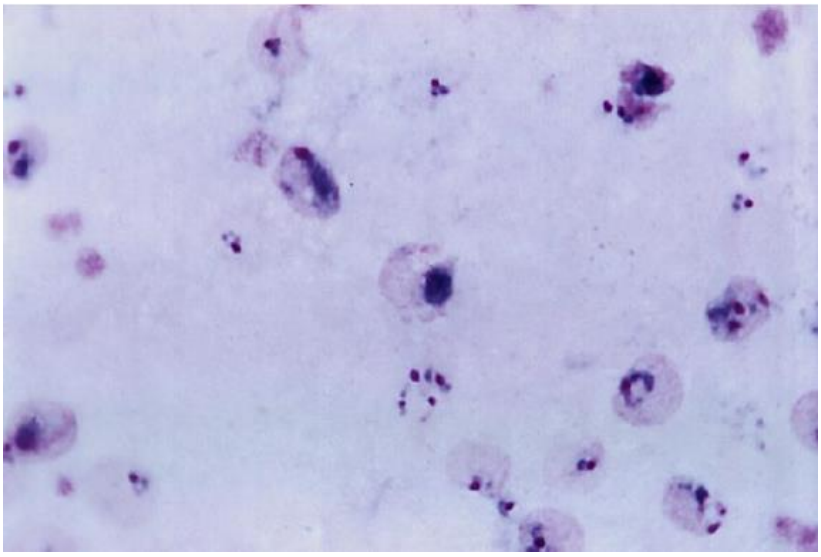
Few to moderate numbers of parasites are present unlike the very large numbers often seen in Pf infections.

Occasionally more than one ring form and double nuclei are seen in red cells, but this is not seen as commonly as it is in falciparum infections.

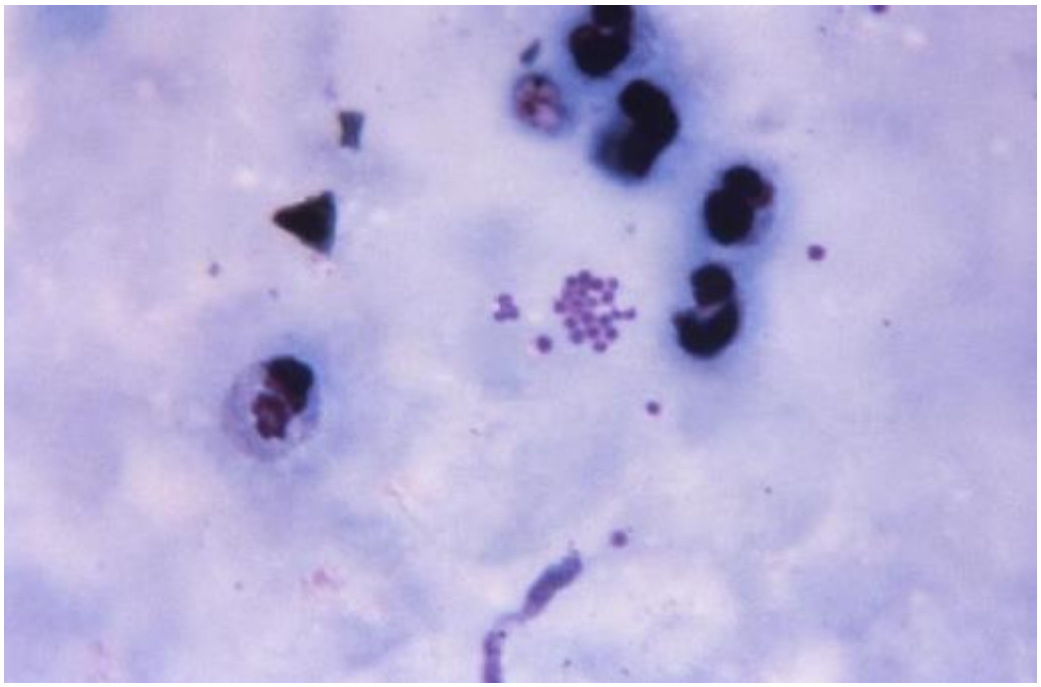
The gametocytes are usually large, round or irregular, and when mature they almost completely fill the red cell. Small forms may also be present.

Schizonts also large round or irregular, filling the RBCs when mature. They are often ruptured in thick films and reveal 16 to 24 or more merozoites.

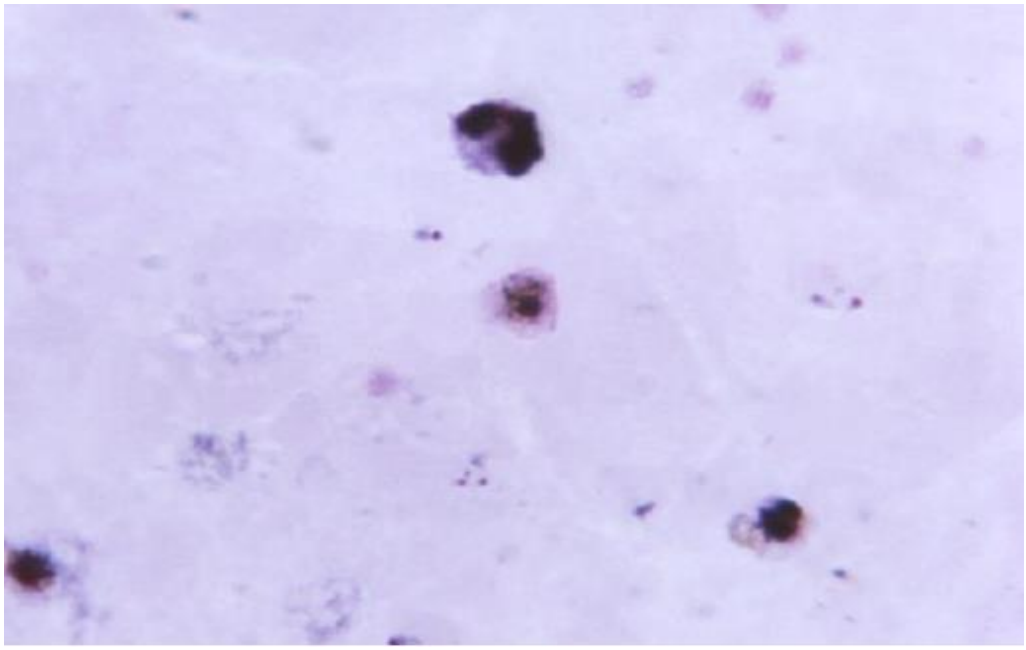
Thick films of P.vivax



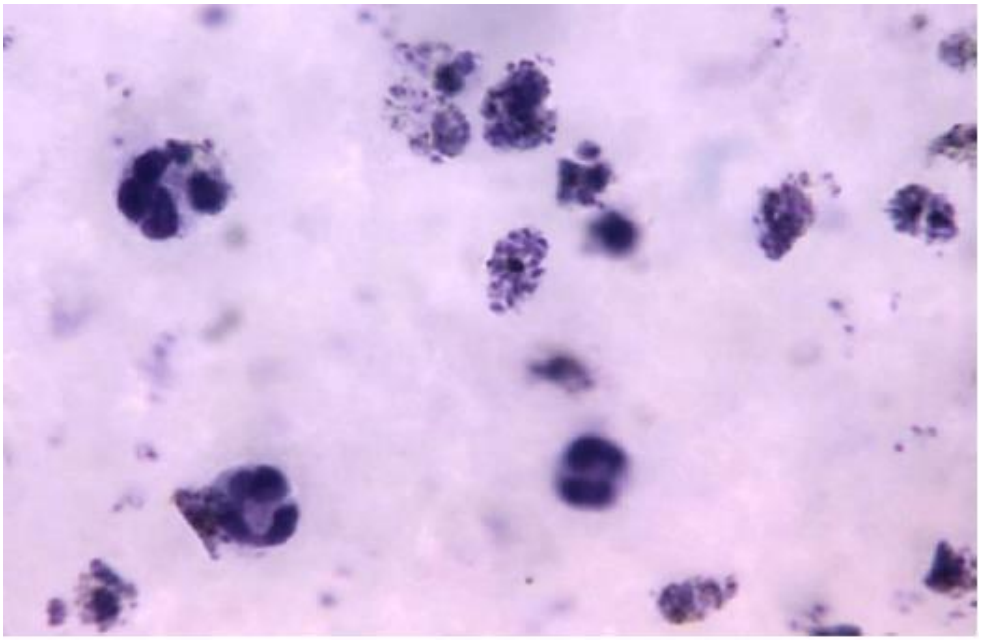
Thick film showing Pv rings and growing trophozoites with Schüffner's dots.



Artefacts including a platelet clump. No parasites are present.

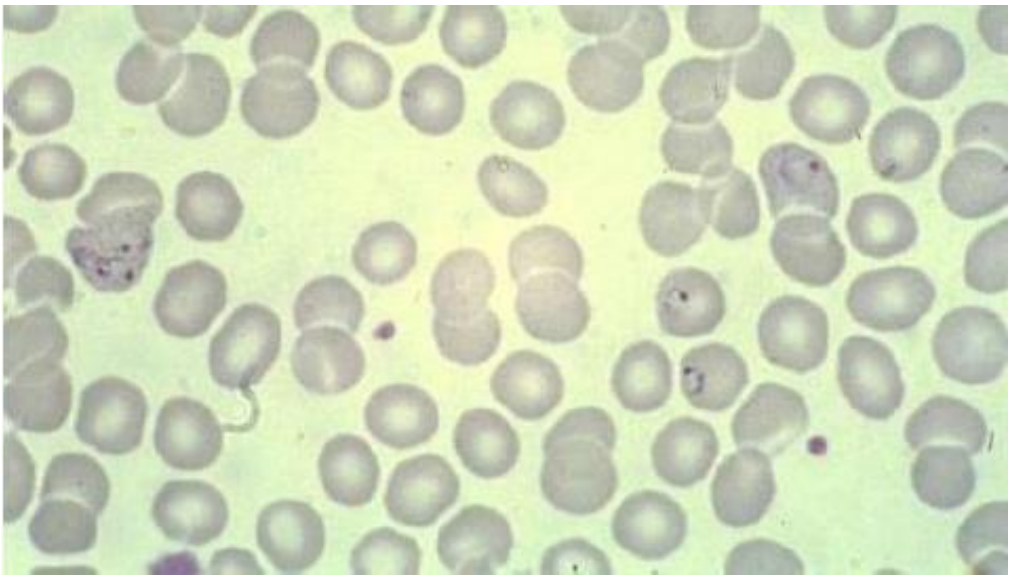


Pv gametocyte and small trophozoite.

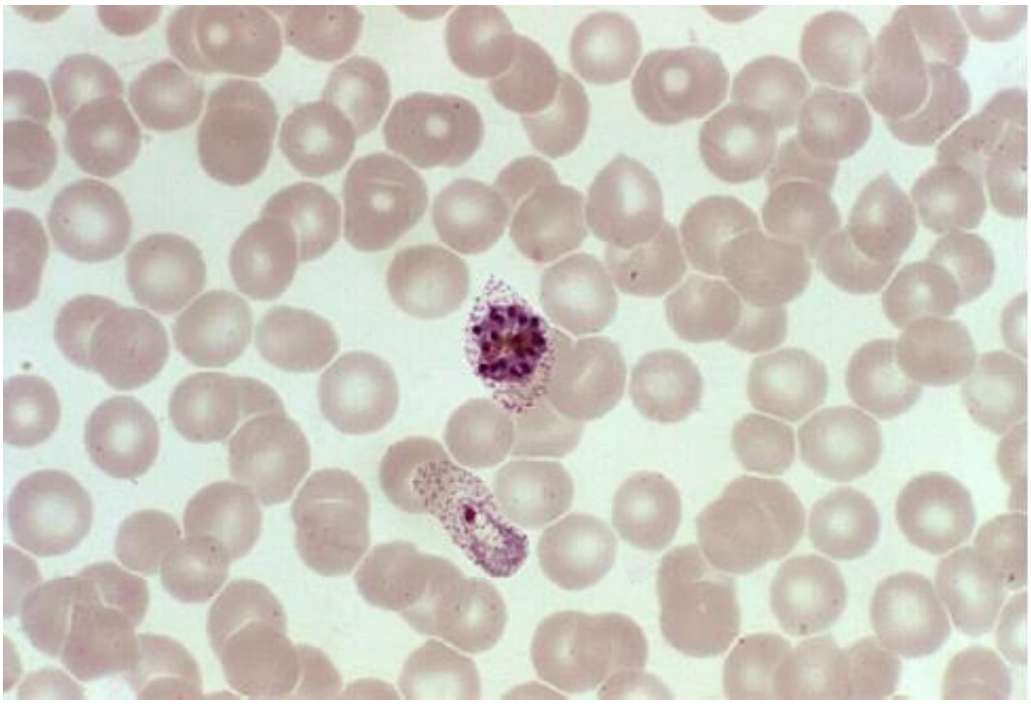


Pv trophozoites and schizonts. Mature schizonts may contain 24 or more merozoites. Note the pigment granule in the RBC above centre.

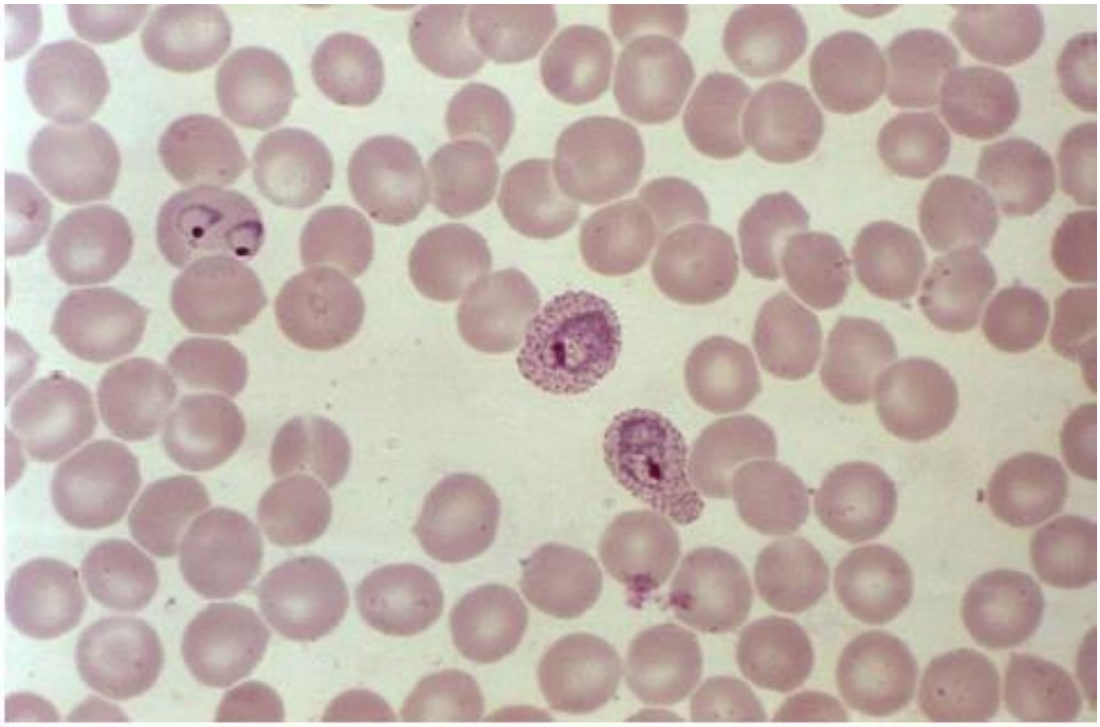
Thin films of P.vivax



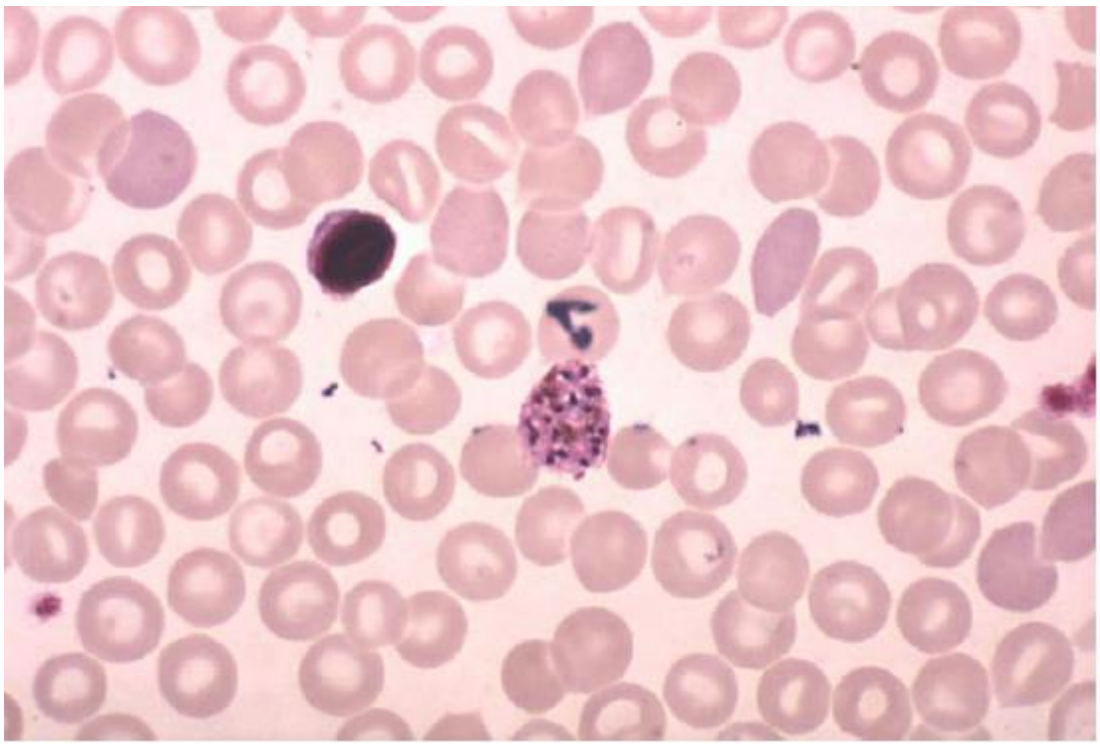
Thin film showing young Pv trophozoites, two early, and two growing rings with RBCs increasing in size and amoeboid shape. Note also the Schüffner's dots



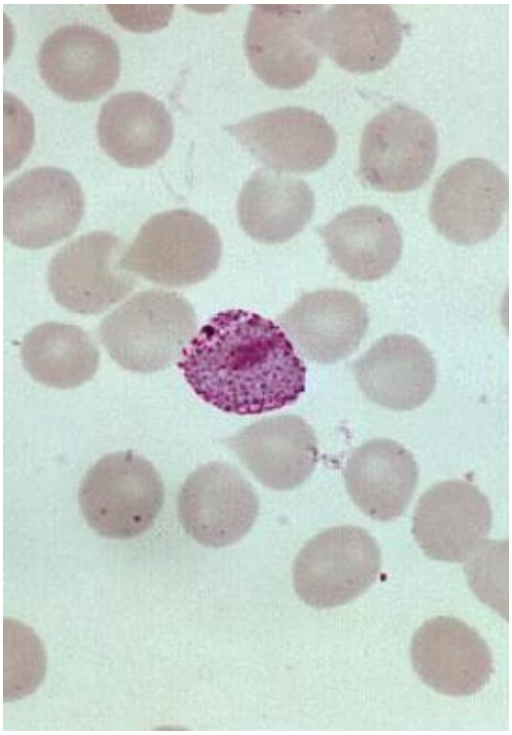
Larger Pv trophozoite and schizont showing clear Schüffner's dots.



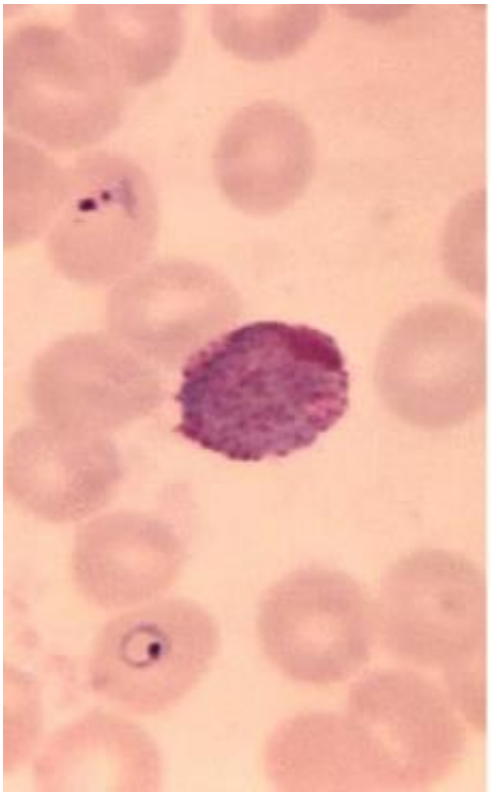
This film shows two Pv rings in one enlarged RBC and two amoeboid growing trophozoites with Schüffner's dots.



Pv mature schizont.

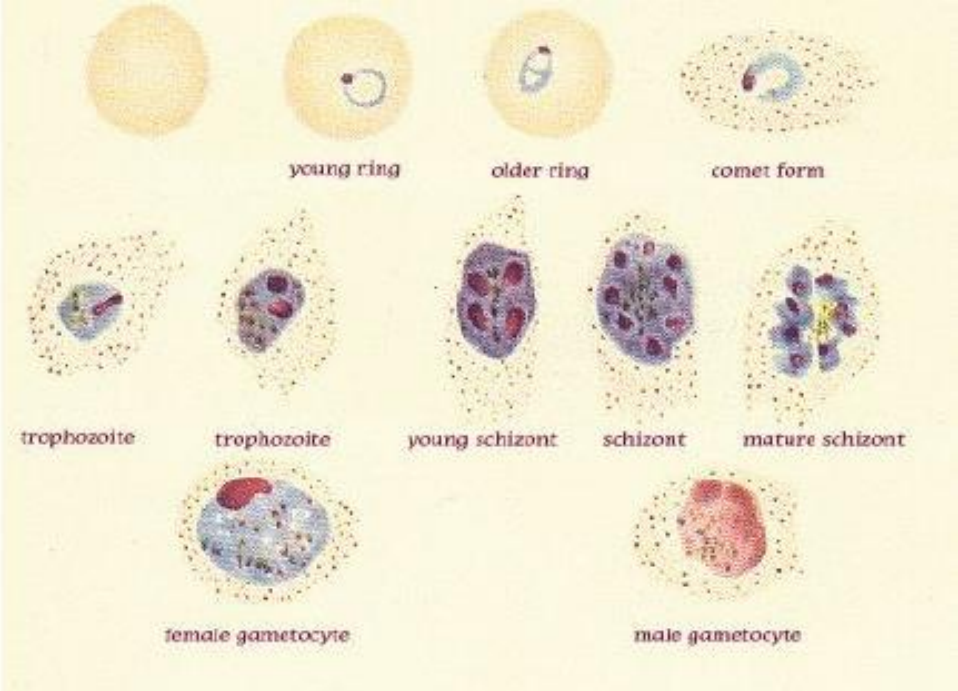


Pv microgametocyte.(male)



Pv macrogametocyte. (female)

P.ovale

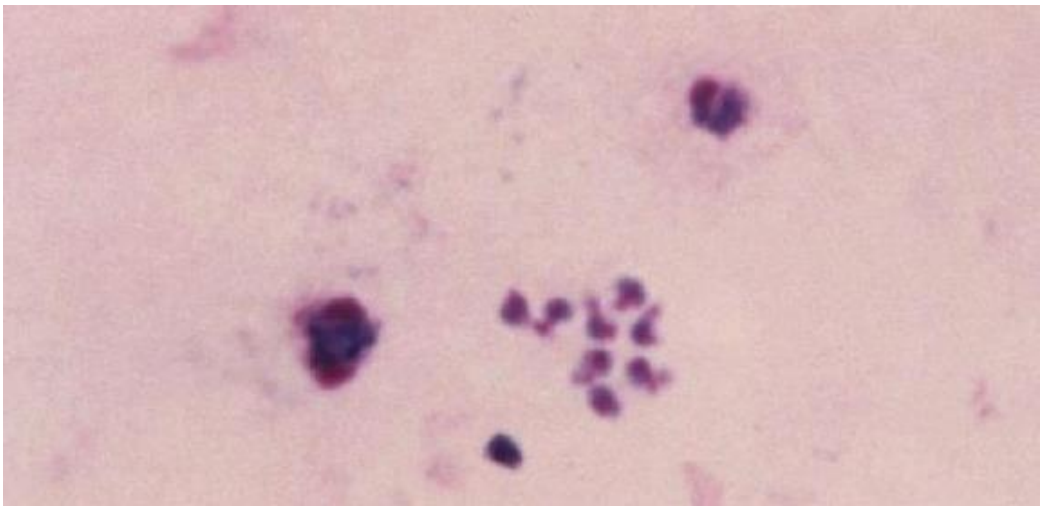


This diagram shows the developmental stages of Po. This species is rarely found in Southeast Asia. All stages of trophozoites, schizonts, and gametocytes can be found in blood films. Usually no more than 2% of RBCs are infected. Occasionally more than one parasite found in RBCs.
(Previous page)

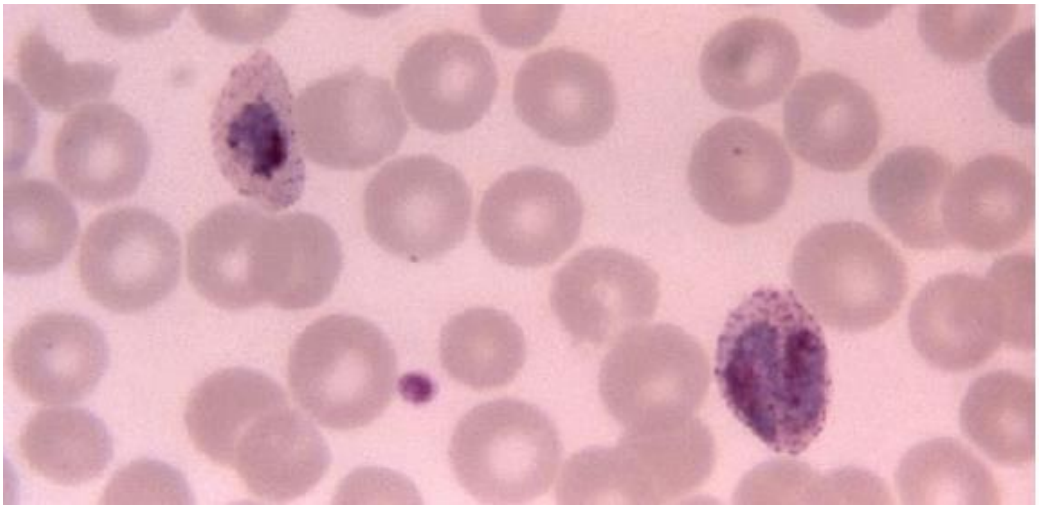
Identifying features of P.ovale

As with Pv, red cell size increases as parasites grow and matures. However, the parasites are smaller than those of Pv and do not occupy the whole host RBC. Up to about 30% of infected RBCs become oval shaped or have fimbriated (jagged) ends. As trophozoites grow, James' dots appear. They are similar to the Schüffner's dots of Pv, but more obvious. They are found in both thick and thin films.
The gametocytes are small and usually round, with James' dots.The schizonts are small with up to 10 merozoites and James' dots. The RBCs are often oval with jagged ends. Only small amounts of malaria pigment may be seen, mainly in the schizonts.

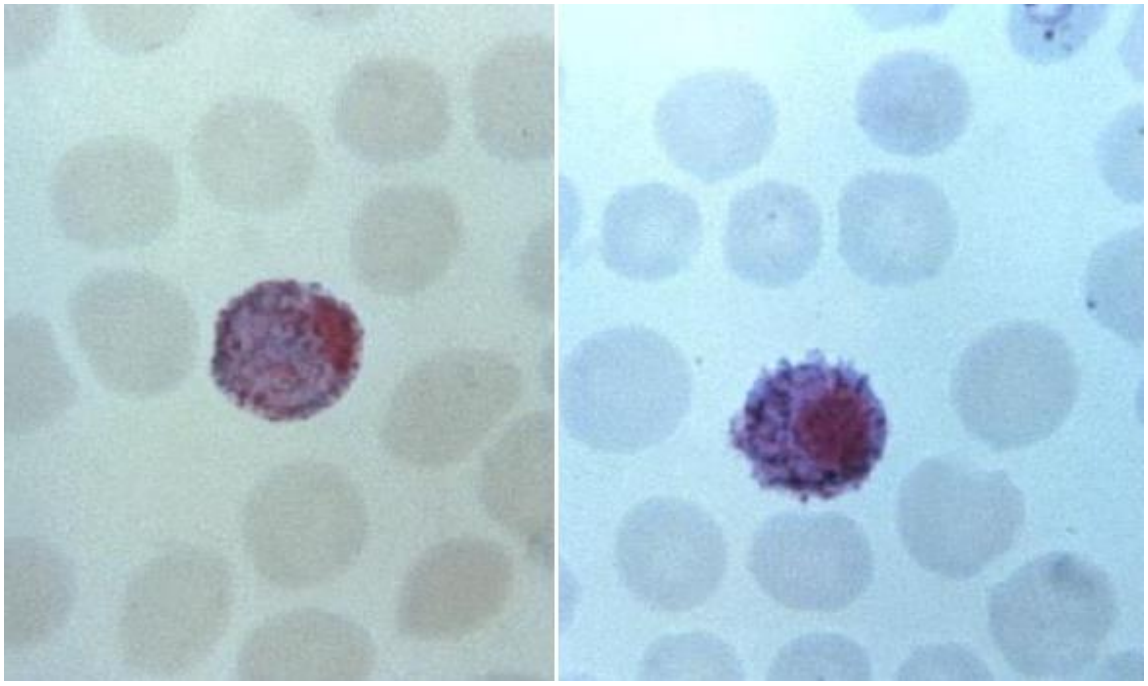
Thick and thin films of P.ovale



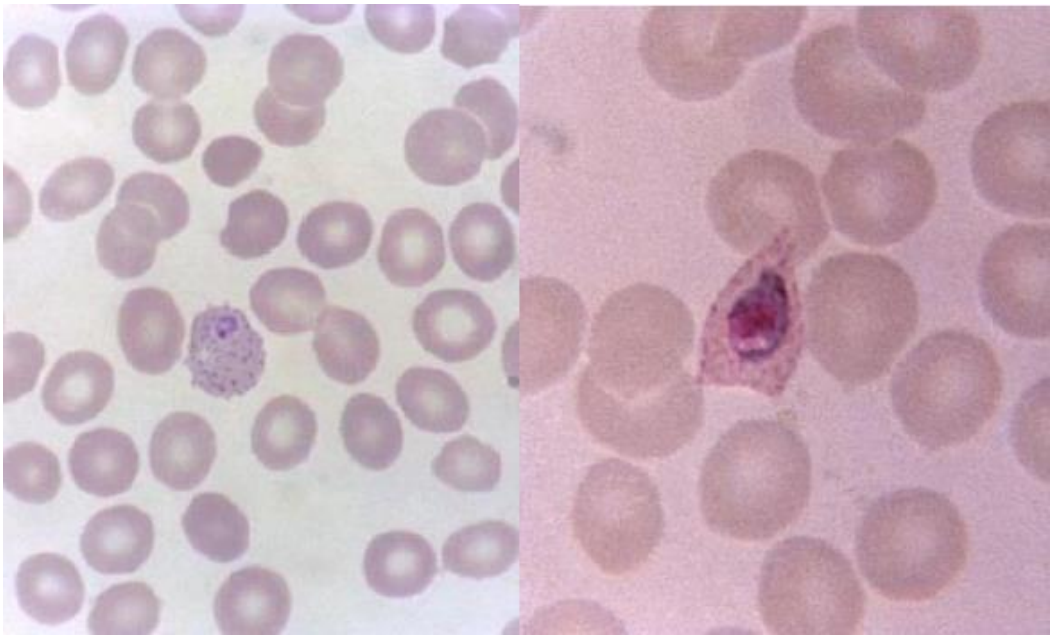
Thick film showing Po schizont with 8 separated merozoites and a growing trophozoite above. The trophozoite shows a large red blood cell outline. An immature schizont is also seen with two chromatin masses. The parasites are smaller than those seen in Pv



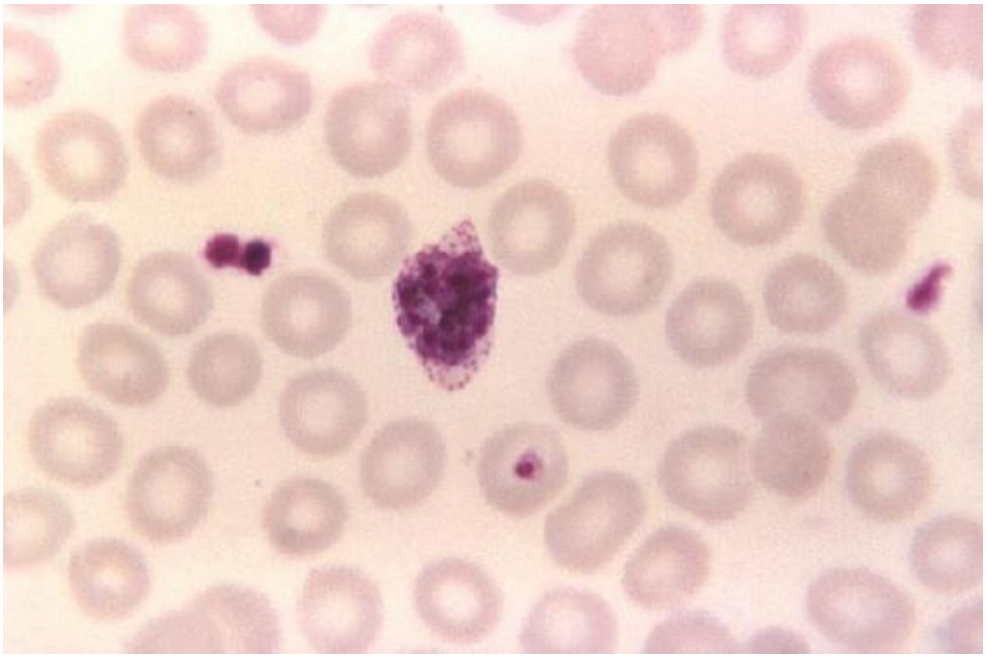
Thin film, Two RBCs with growing Po trophozoites and James' dots. One RBC has two parasites.



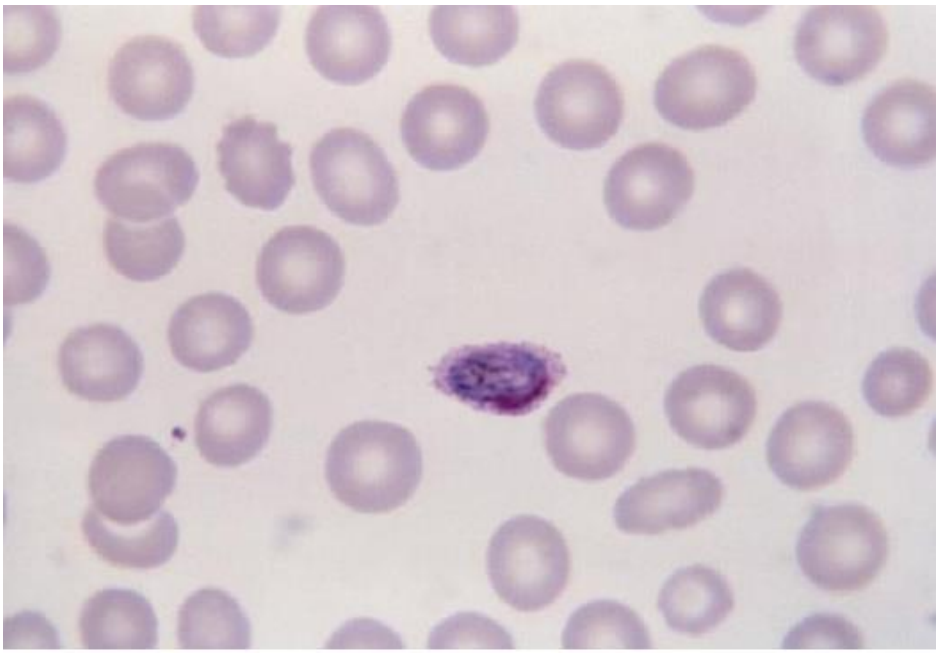
Thin films showing a Po microgametocyte (male) on the left, and a Po macrogametocyte (female) on the right.



Thin films showing Po early trophozoite on the left, and Po growing trophozoite on the right.



Po schizont in thin film.



Po mature trophozoite. This can be confused with the Po gametocyte

P. malariae



This diagram shows the developmental stages of P. malariae. This parasite is only sometimes found in Southeast Asia.

Identifying features of P. malariae

P malariae: All stages of trophozoites, schizonts, and gametocytes are found in the blood films. Rarely more than 1% of RBCs are infected.

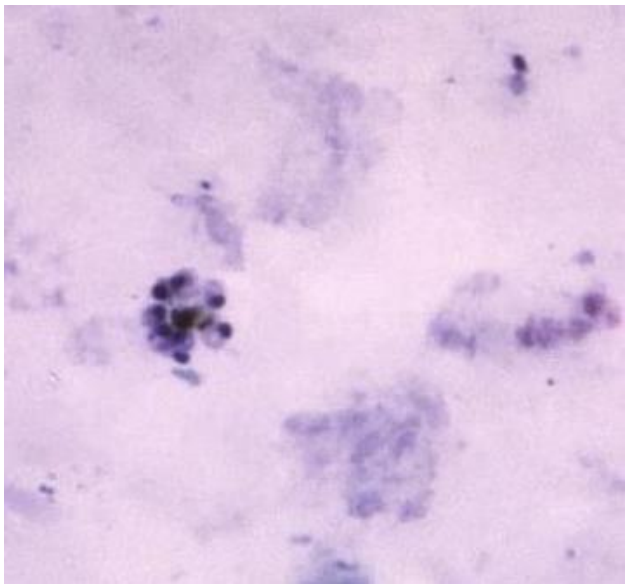
RBCs are not enlarged and do not contain dots. Ring forms, band forms and basket forms of trophozoites may be present. Malaria pigment may be present in growing trophozoites. Mature trophozoites appear similar to gametocytes.

The gametocytes are small, round or oval, with yellow brown malaria pigment. The nucleus usually lies to one side.

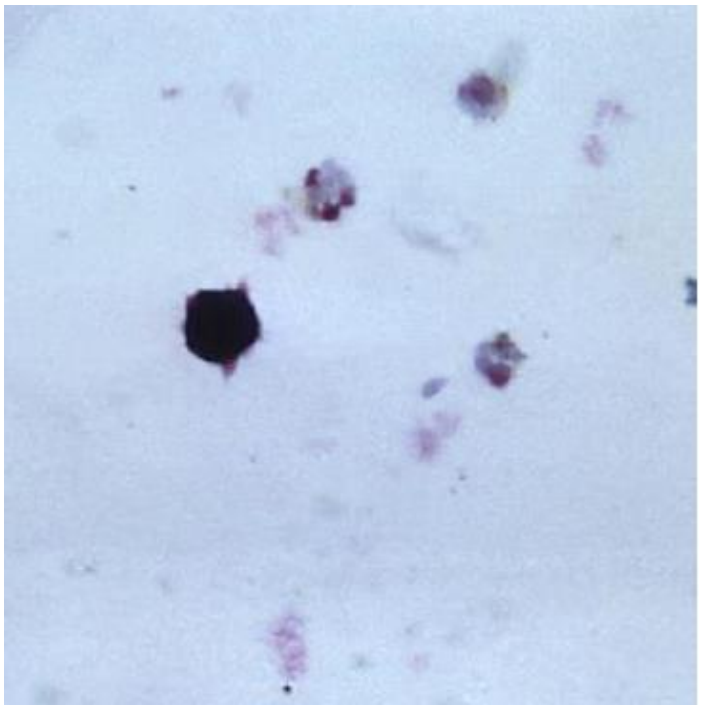
The schizonts are small with up to 12 merozoites and malaria pigment.
Mature schizonts often have merozoites neatly arranged around malaria pigment to give a daisy like appearance.

(trophozoites, schizonts,♂♀gametocytes)

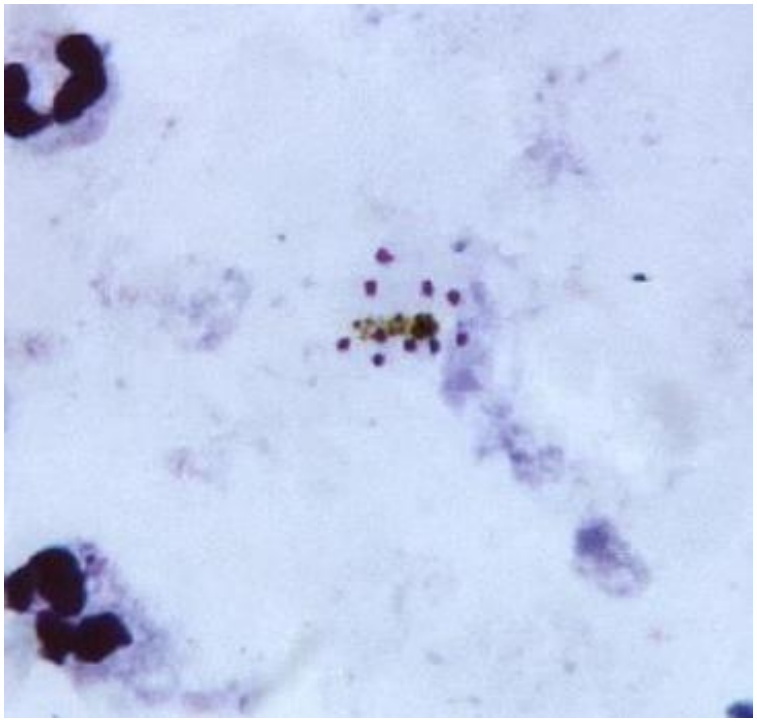
Thick films of P malariae



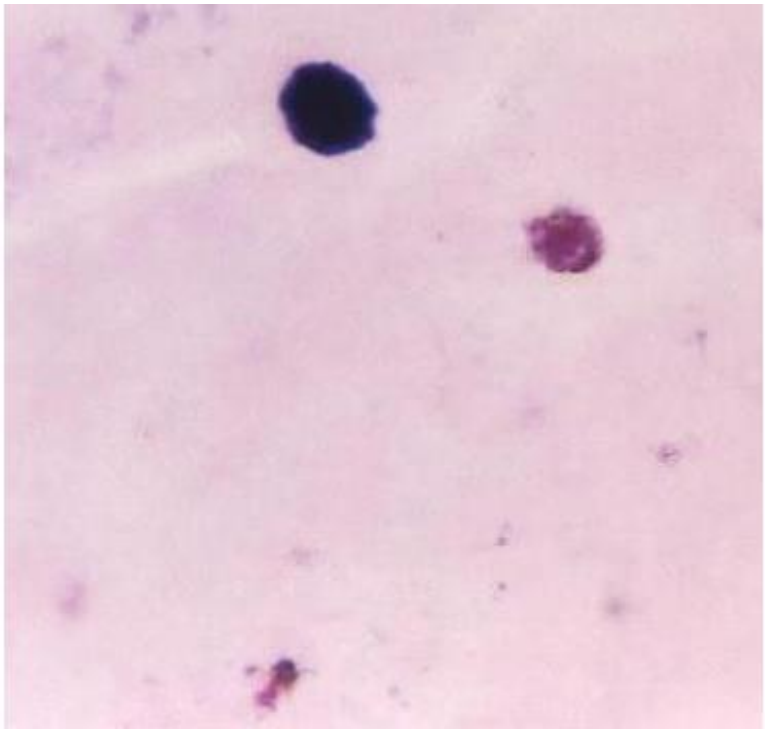
Thick film showing a Pm schizont and early trophozoite.



Pm late trophozoites and developing schizont.

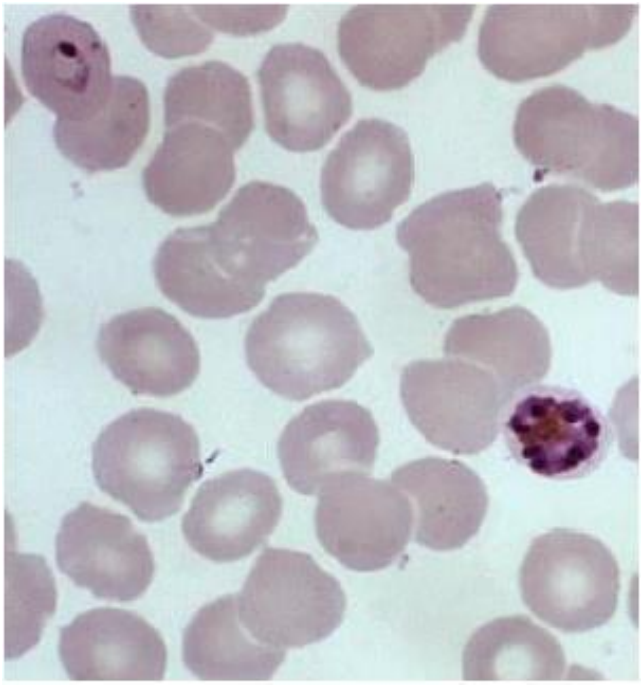


Thick film showing ruptured Pm schizont with merozoites and malaria pigment.

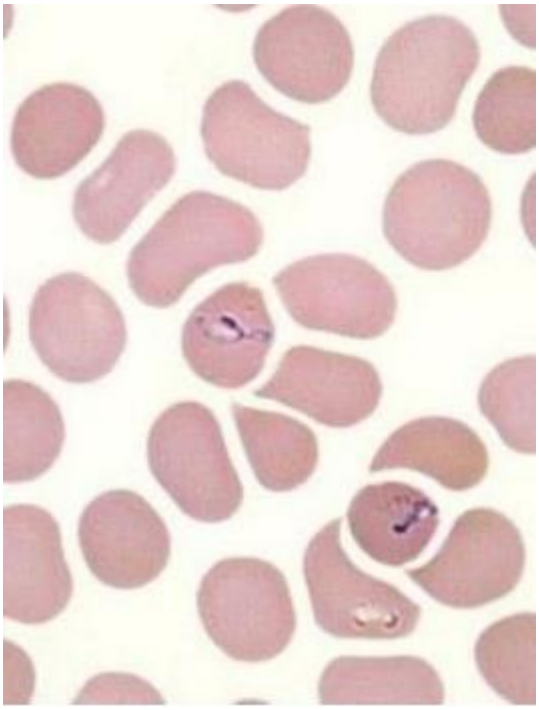


Pm gametocyte and trophozoite.

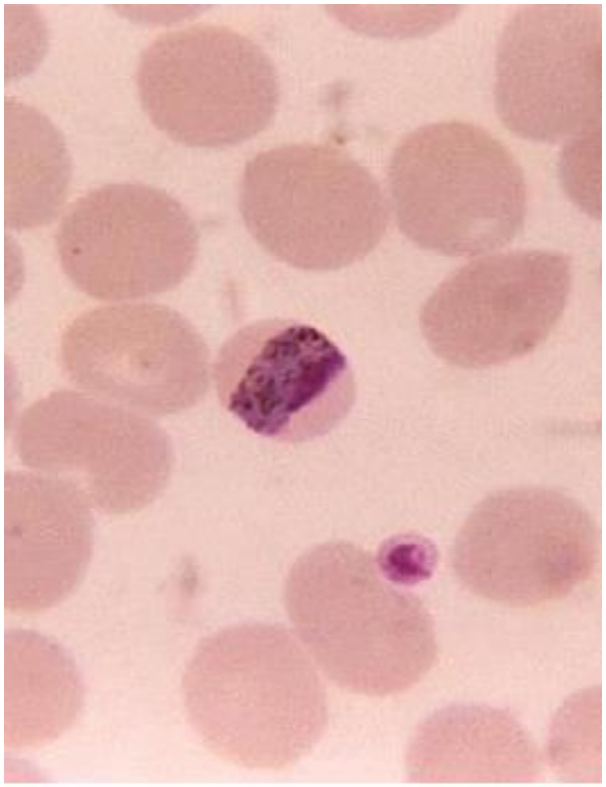
Thin films of P. malariae



Thin film showing Pm early ring trophozoite and schizont

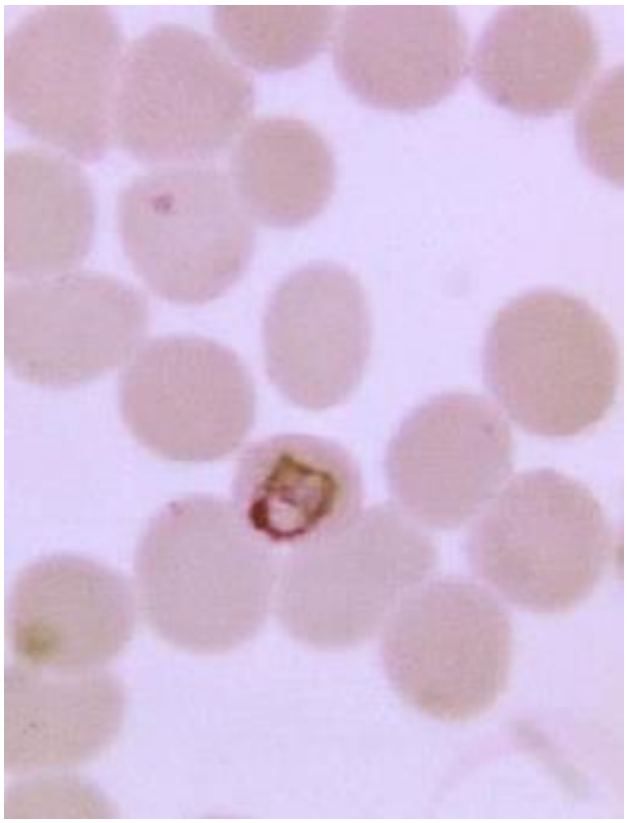


Pm band form early trophozoites.

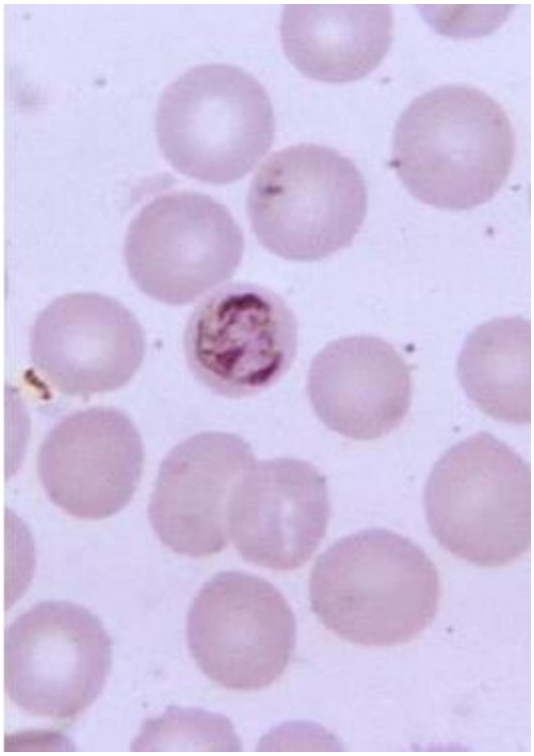


Pm late trophozoite band form.

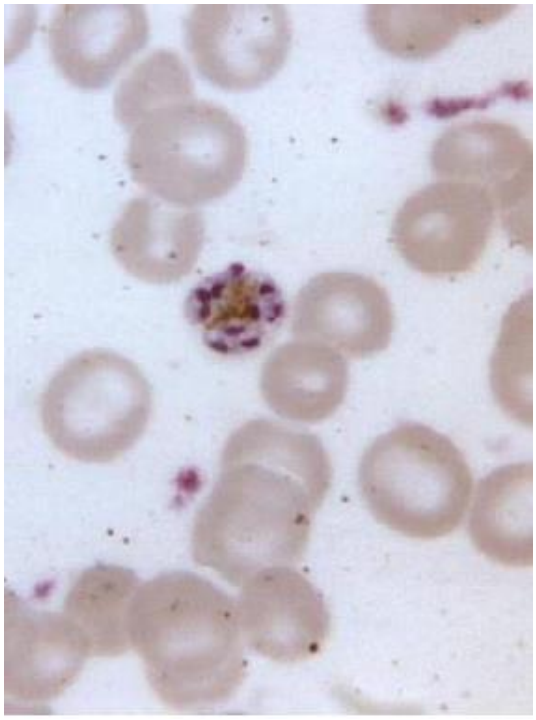
.



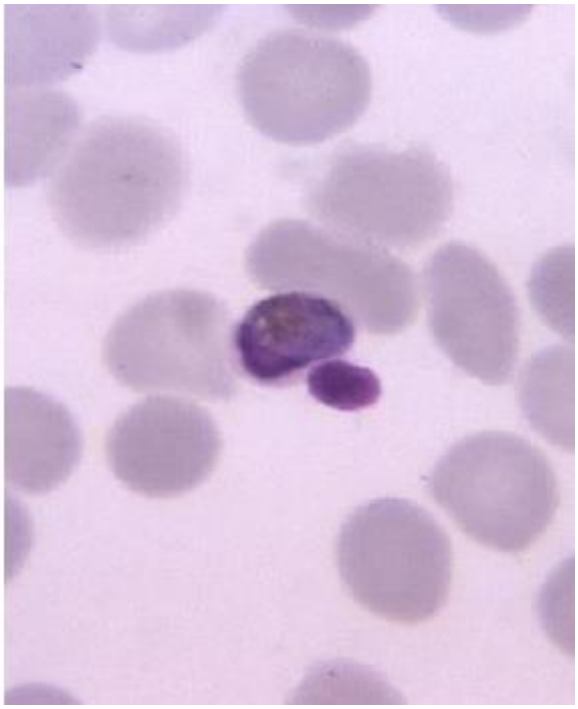
P. malariae basket form trophozoite.



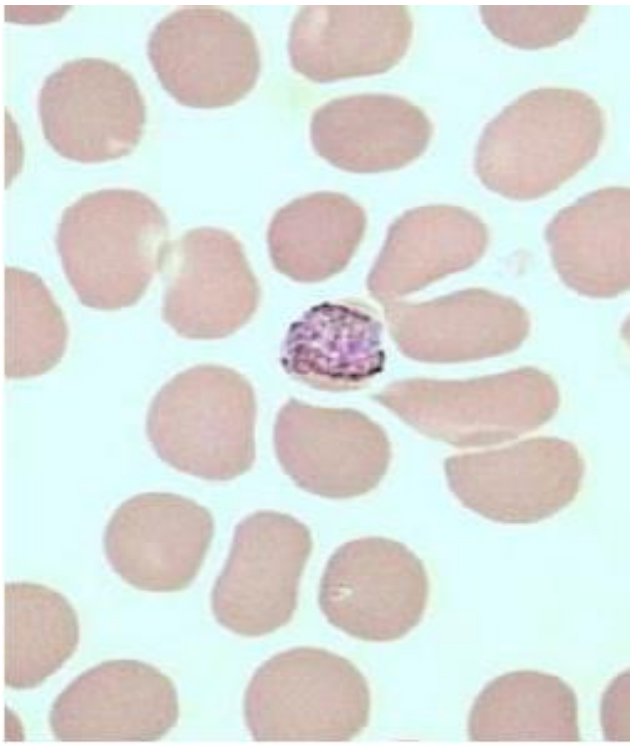
Thin film showing Pm developing schizont.



Pm mature schizont.



Pm late trophozoite.



Pm gametocyte. Do not confuse with Pm trophozoite, previous page.

P. knowlesi

This is a Malarial parasite of monkeys that can also infect humans. It is mainly in Malaysia and causes a serious and life threatening disease. In some areas it accounts for half the human malaria. Sometimes it is found as a mixed infection with Pf. This species of Plasmodium has been found in Thailand and could become a problem in Burma as forests are cleared and humans have closer contact with monkeys.
Due to similarity in appearance it can be wrongly diagnosed as Pm. In this infection many RBCs are infected and all stages may be present in smears at the same time. The blood cycle is 24hours with fever recurring daily.

Identifying features of P. knowlesi

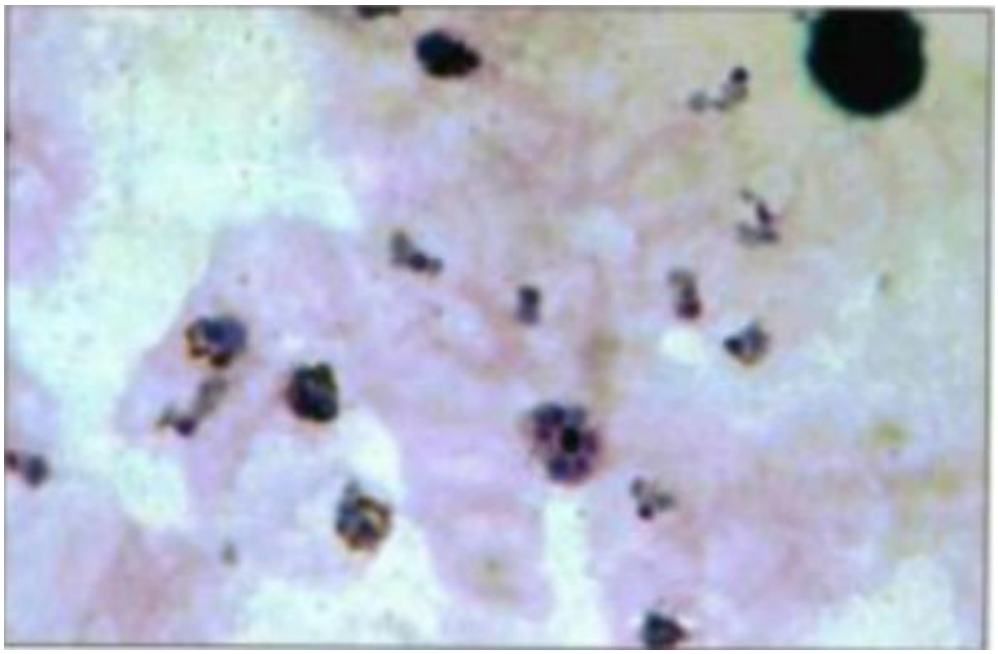
RBCs are not enlarged. They may have fimbriated ends and may contain small dots called Sinton stippling.

The trophozoites may look like Pfrings if only early forms are present. There may be more than one parasite and some with double dots in RBCs. The cytoplasm is compact and band and basket forms like those of Pm may be found.

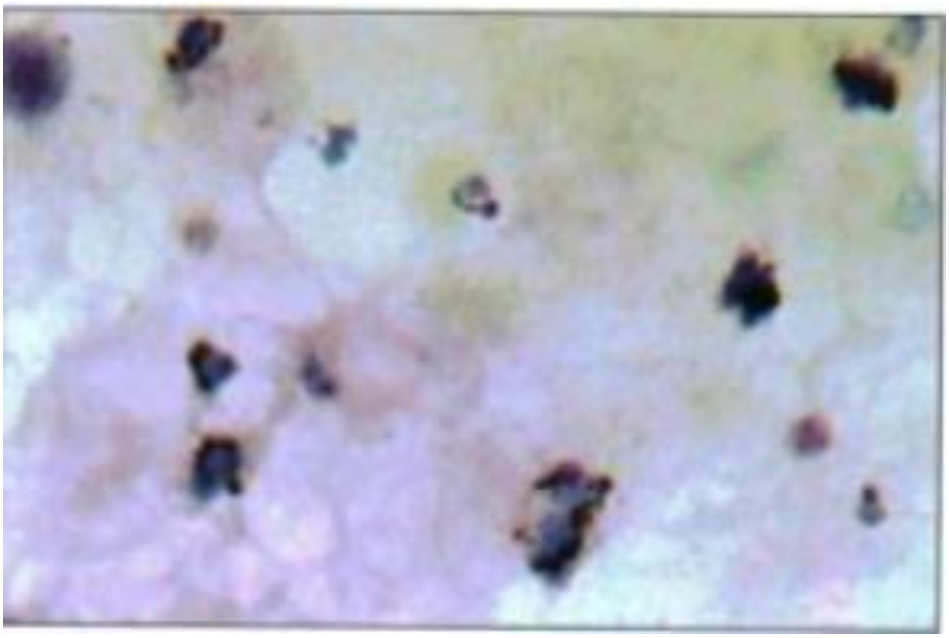
The schizonts resemble those of Pm with malaria pigment. They contain up to 16 merozoites.

The gametocytes are also round with scattered malaria pigment. They tend to fill most of the Red blood cell. The overall appearance is similar to that of the Pm gametocytes.

Thick films of P knowlesi

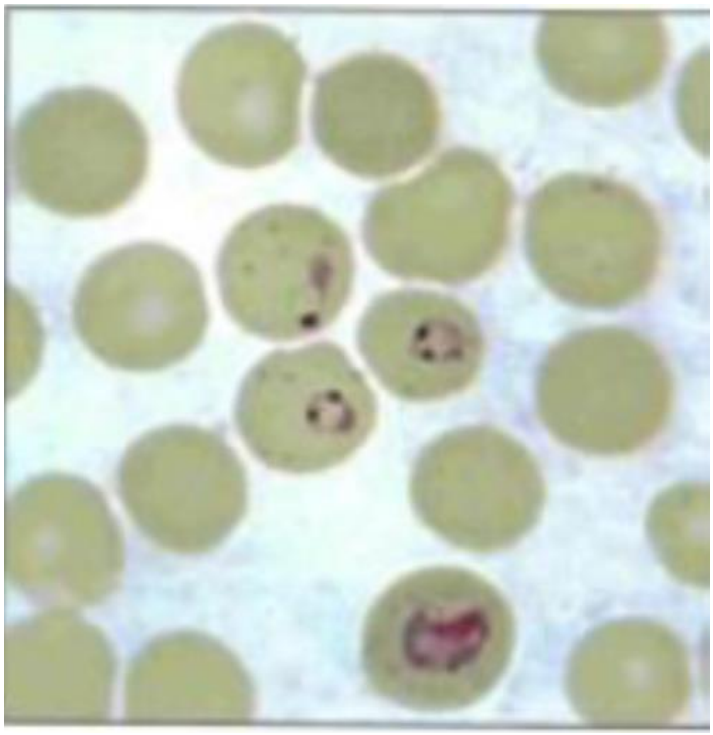


Thick film showing a heavy infection of Pk with trophozoites, gametocytes and schizont.

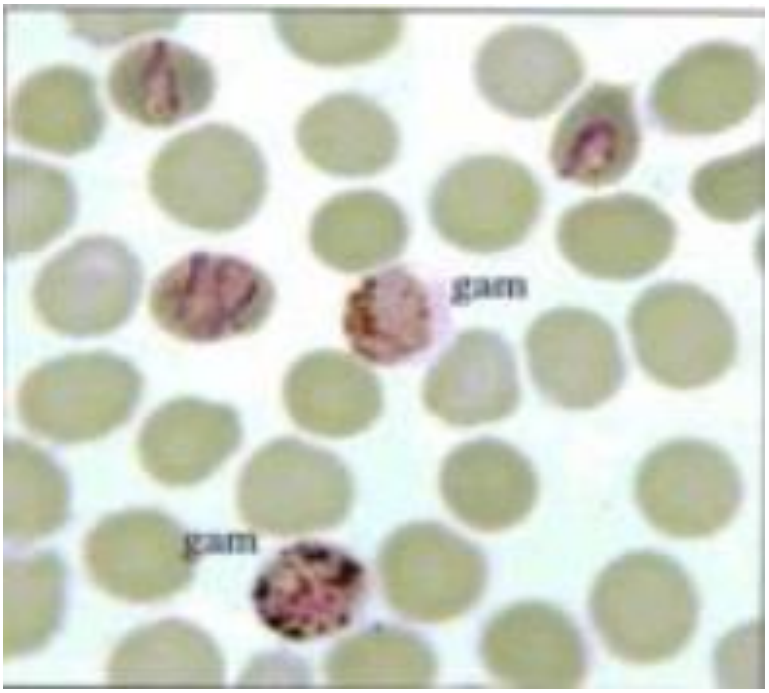


Pk trophozoites and gametocytes.

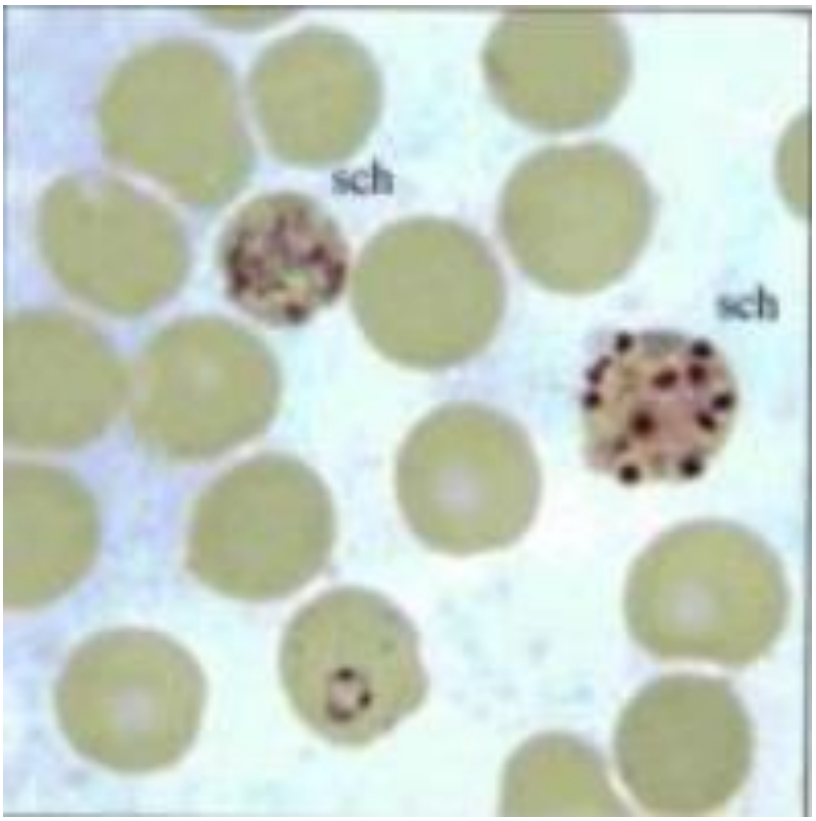
Thin films of P. knowlesi



Pk trophozoites, some with dots and a basket form.



Pk gametocytes and band trophozoites.



Thin film showing Pk schizonts and a trophozoite.

Part Two

Preparation and Staining of blood smears for Malaria diagnosis.



Items required for capillary blood sampling for various tests.



Extra items for malaria smears.

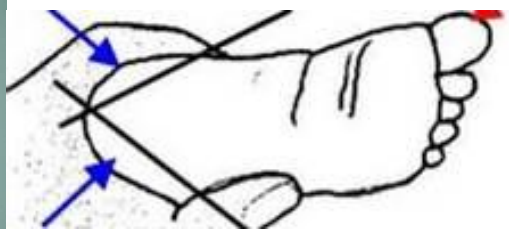
We prepare thick and thin films of blood, on a microscope slide,
for diagnosis of Malaria.
Have these things ready for use:

New microscope slides. Also a special smooth, clean ended slide for spreading thin film.
Cotton wool balls.
Alcohol or Alcohol wipes for skin preparation.
Gloves.
Blood lancets.
Bin or bag for disposable waste.
Sharps container for used blood lancets. This can be a drink bottle with lid.
Pencil
Record book and pen.

Clean one of the slides, with a tissue, holding it by the edges: Never touch the slide surface !
Also have a clean ended spreader slide ready to use.
Put on the gloves.

The following pictures show sites for pricking with lancet to obtain blood sample. Use the hand not used for writing in adults. In infants and small children use sides of heel, big toe, or thumb.





Clean puncture site with alcohol on cotton wool, or an alcohol wipe.

Open the blood lancet paper, (or plastic cover) to expose the sharp end.

Prick the finger or other site firmly and rapidly.

Immediately place the used lancet in the sharps container for proper disposal.

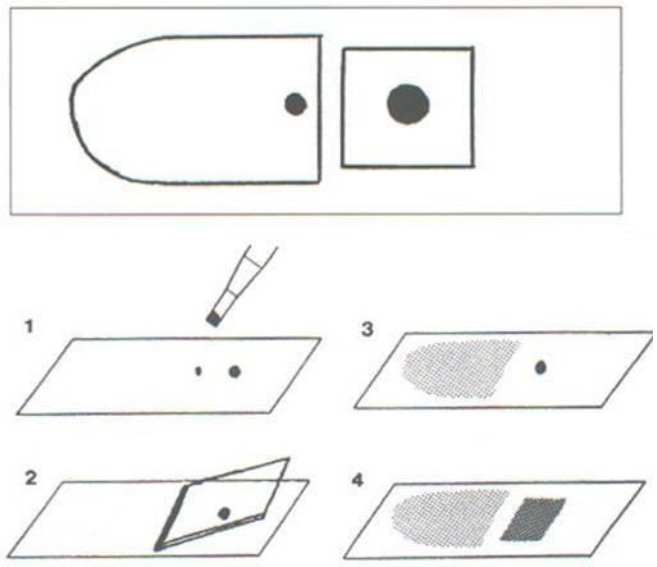
With a dry piece of cotton wool, gauze, or tissue, wipe away the first drop of blood.

Put this and all other disposable waste in to bin or bag as it is used.

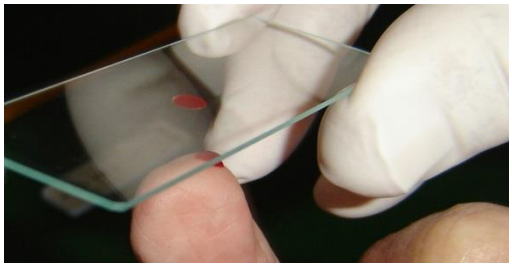
Preparation of Thick and Thin smears:

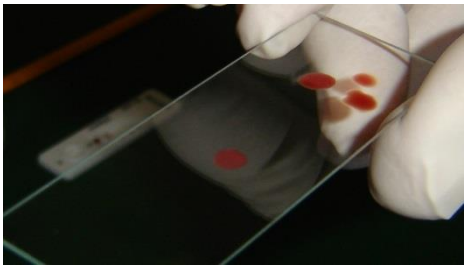
Squeeze finger or other site gently to obtain freely flowing blood for making slides.

When applying the blood on the slide, touch blood only, not finger.

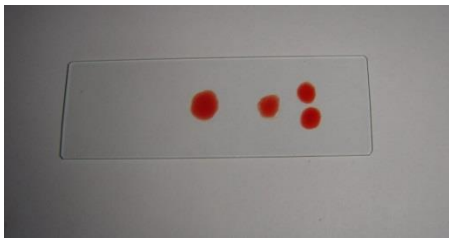


Slide card for showing size of blood drops and area of slide to cover for thick and thin films,
and diagrams showing making slides, and ideal size of films.
We use a 1 cm round thick film. (this is easier to make than the square one shown here).



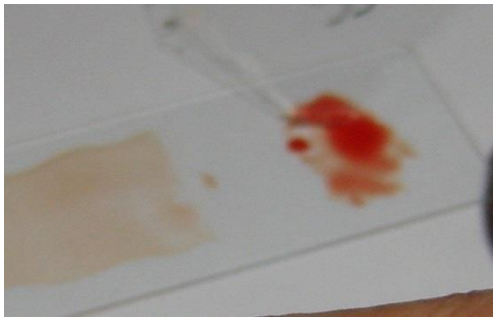


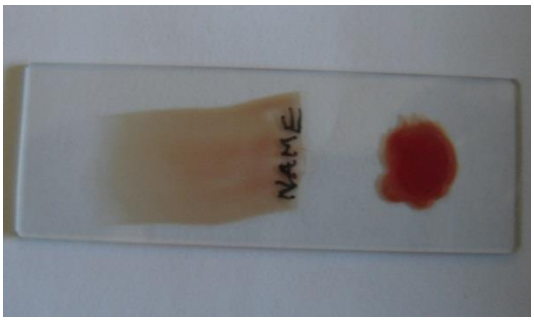
Place one small drop of free flowing blood in middle of test slide as shown for thin film.
Place three smaller or one larger drop between this and one end of slide for thick film.





Place slide on a flat surface. Hold the spreader slide against the blood slide at a 45 degree angle and draw it back to touch the drop of blood for the thin film, the blood will run across the end of the spreader. Keeping the spreader firmly on the slide, make the thin film with one smooth forward movement to the end of the slide.
Immediately use the corner of the spreader to spread the blood for the thick film. Do this with three or four rotary movements only to form a round film about 1 cm in diameter. More mixing will cause clumping of RBCs. It should be possible to just see, but not read, newsprint through the thick film.





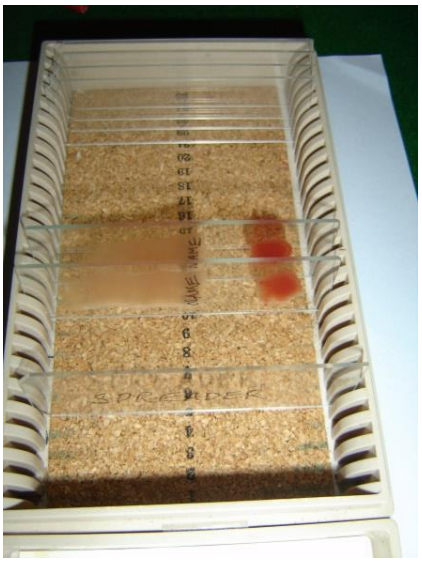
Allow slides to dry at room temperature. Write patients name or number and date with pencil across the thickest end of the thin film.

The quantity of blood is very important for the thick smear! Not enough blood may lead to a false negative result. A smear with too much blood will not be stained properly and cannot be used.

<u>Fixing the thin film:</u>

Hold each slide on an angle with thick film uppermost, run a few drops of methanol, from a tightly sealed dropper bottle, over the thin film only to fix the RBCs. Do not allow Methanol to contact the thick film. Stand slides this way up for a few minutes to completely dry.

Slides can now be stained with diluted Giemsa stain, or stored covered in a slide box or tray to be sent for staining.in a laboratory.



Staining

Materials needed:

Pure Giemsa stain. It may be necessary to filter the Giemsa stain before using it (depending on the quality of stain), if so, shake the original bottle of pure Giemsa stain first, then filter it, using filter paper and funnel, into another clean dry brown glass bottle and tighten the lid.
Giemsa contains methanol , containers must always be tightly sealed.

Clean filtered water with pH at or near 7.2

Wash-bottle or beaker.

Graduated cylinder (100 ml)

Graduated cylinder or pipette(10ml), or plastic disposable pipette, or syringe

Slide-rack

Staining tray

Giemsa stain must be diluted with water before staining slides. We use a 10% dilution in pure water, filtered if necessary, for 20 minutes staining time. The pH of the water should be at or near
7.2. If it is less than pH 7.0 the staining will be too pink, over pH 7.4 it will be too blue. This can be corrected by adding pH 7.2 buffer tablets or solution to the water used for stain dilution.

Stain dilution:

For a batch of 20-30 slides:
Measure 90 ml water as above in to 100ml cylinder.

Add exactly 10 ml of filtered Giemsa into the water by using dry pipette or cylinder.
Pour into a clean and dry wash bottle or beaker.

For smaller batches of slides prepare only the amount of stain required, allowing about 4 ml of
diluted stain per slide. eg.
1 ml Giemsa to 9 ml water in a 10 ml cylinder for 1 or 2 slides.
5 ml Giemsa to 45 ml water in a 100ml cylinder for 10 to 12 slides.

Staining:

The staining rack can be a cooking grill over a slightly smaller size plastic container as in picture, these can usually be found in local shops or market.



Lay slides to be stained, blood film side up, on the flat staining rack.
Start timing and gently cover the slides with diluted stain from beaker or wash bottle.
Let the solution remain on the slides for 20 minutes.
Wash slides with clean water following the same order as for the staining.
Pour the water, gently, over the slides from the thin smear-end, keep them lying flat on the tray until all stain is washed off, to prevent stain debris on films.
Then tip them to pour the water off and stand them to dry with thin film upwards.

Microscopic examination can be started as soon as the thick film is completely dry, or any time later.

Keep the stained slides covered in a slide box or tray before and after staining. Most of the immersion oil can be removed before storage by wrapping them in toilet paper or tissue and leaving them for a few minutes.

All reported slides must be kept and sent regularly to a base laboratory to be checked for Quality Control of results and staining.

All blood film preparation and staining should be done inside or in shade. Do not allow blood films to stand in the sun, and keep them covered to prevent them being eaten by flies etc.

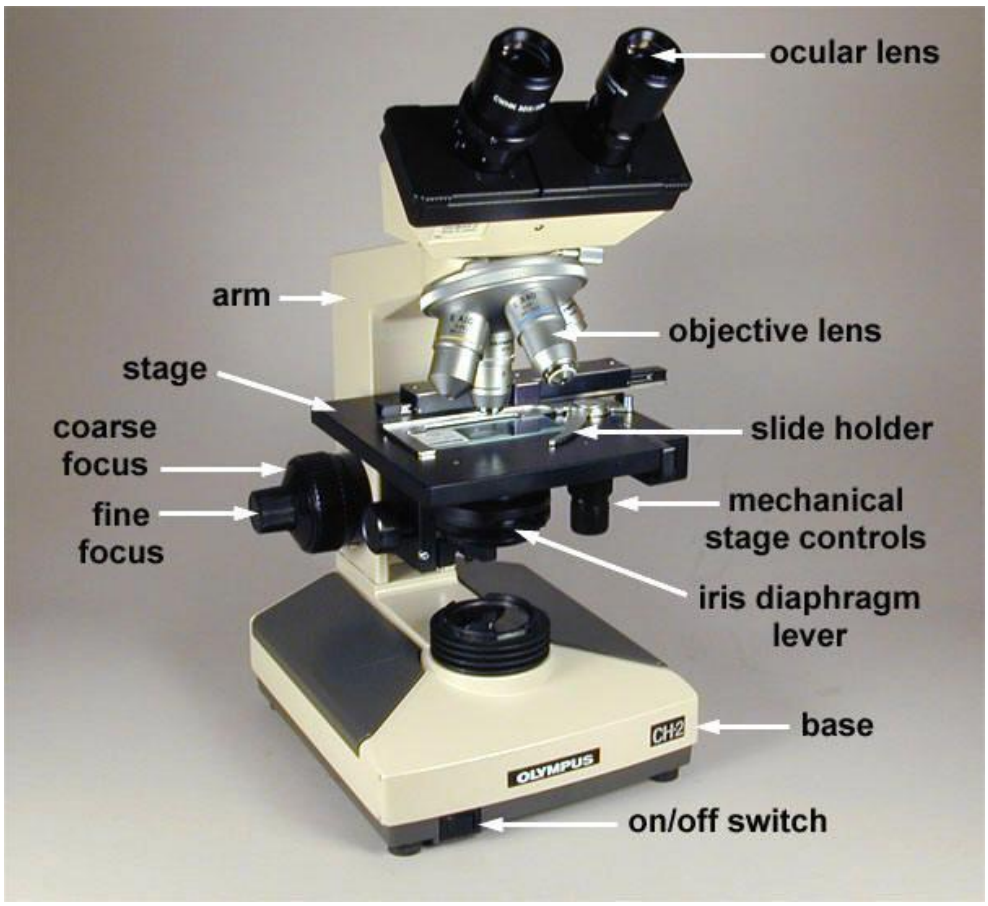
The Use and Care of the Microscope

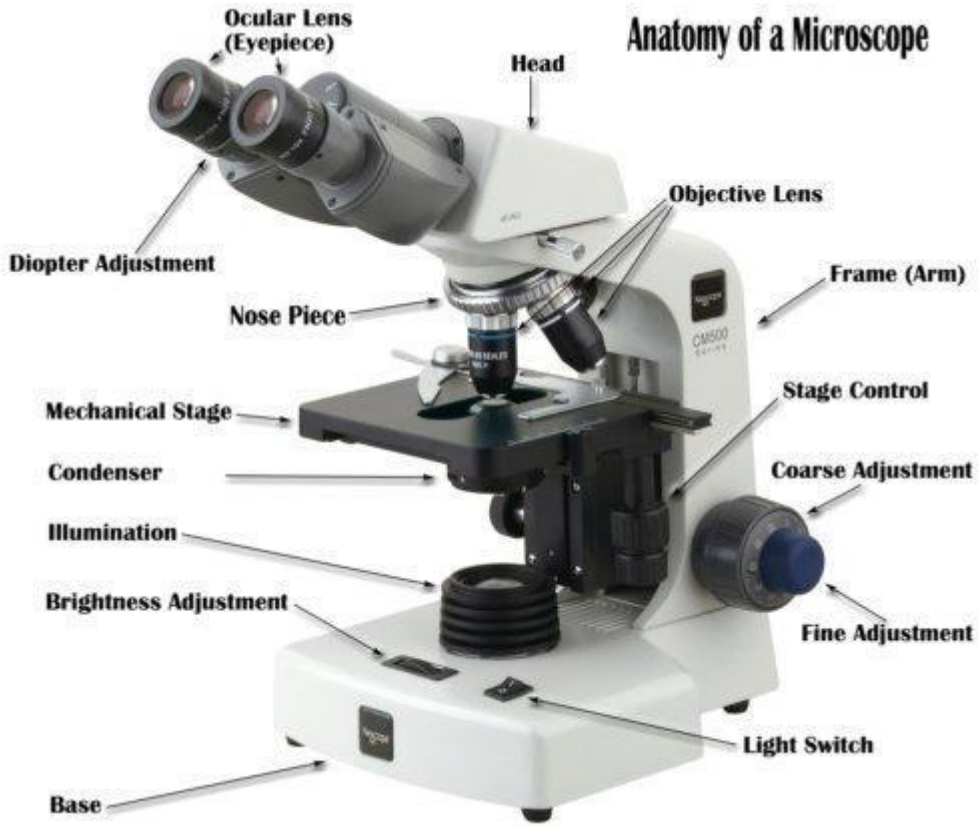
The light microscope is the most important item of equipment for the diagnosis of Bacterial, Parasitic and Fungal infections. It allows us see things too small to see with our eyes by magnifying them up to 1,000 times.

It is a delicate instrument and can easily be damaged and become unusable if not treated with care. These pictures show some of the parts.

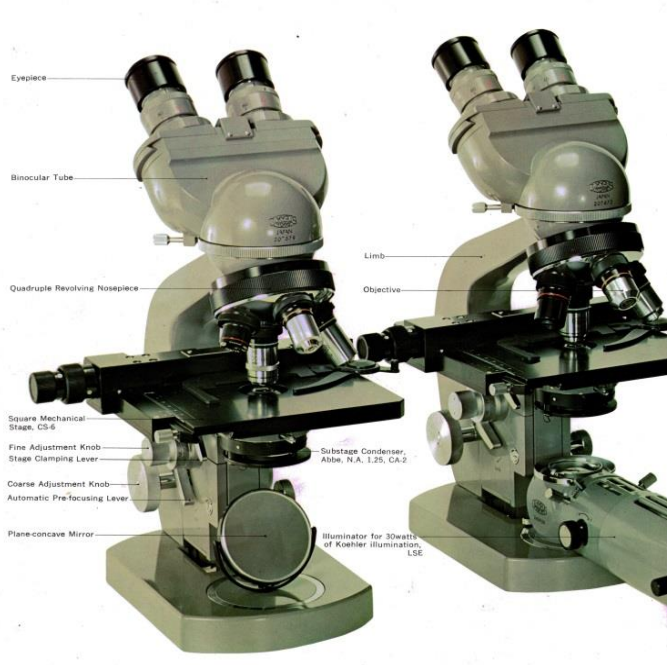
All the optical parts- eyepieces, binocular head, objectives, and condenser, can be destroyed by fungus attacking glass lenses and prisms in humid tropical countries.
This can be avoided by keeping them in air conditioned places, or in a box with an electric light bulb to dry the air.
If there is no electricity they must be kept in an airtight box with an open container of fresh Silica gel to absorb moisture.

Here are examples of binocular microscopes with built in electric light source, an external light source, and a mirror. Sometimes we use daylight or an external electric light with a mirror for illumination.





Microscopes with mirror for daylight and external electric light source usually plugged in to a transformer.



Use the concave side of the mirror for daylight, the flat side for electric light.

When using electric light, a **blue filter** must be used either over the light outlet in the base, or in a holder in the bottom of the condenser. No filter is used for daylight microscopy.

When moving the microscope from its box to the work bench each day, carry it with one hand holding the arm, and the other firmly under the base.

Basic Parts of the Microscope

The Support system

1. The Base.
2. Arm, also called the Body or Frame.
3. The Head that holds the eyepieces.
4. The nosepiece that holds the objectives.
5. The Microscope stage to place a microscope slide or other object.
6. The Mechanical stage for moving the slide while viewing.

The Magnification system

Consists of a system of lenses:

The Eyepieces.

They multiply the size of the object by 10 times.



The Objectives

10X objective multiplies the size of the object 10 times.

40X objective multiplies the size of the object 40 times.

100 objective multiplies the size of the object 100 times

With the100x objective, we use immersion oil on the slide.

Magnification example: If the x 40 objective and the x 10 eyepiece are used, the total magnification will be 40 times (objective) multiplied by 10 times (eyepieces): 40x10 = 400.
It means that the image will be magnified 400 times from natural eyesight image.



Olympus lenses are identified by:

10x yellow stripe,

40x blue stripe,

100x oil immersion white stripe.

LIGHT PATH PARTS

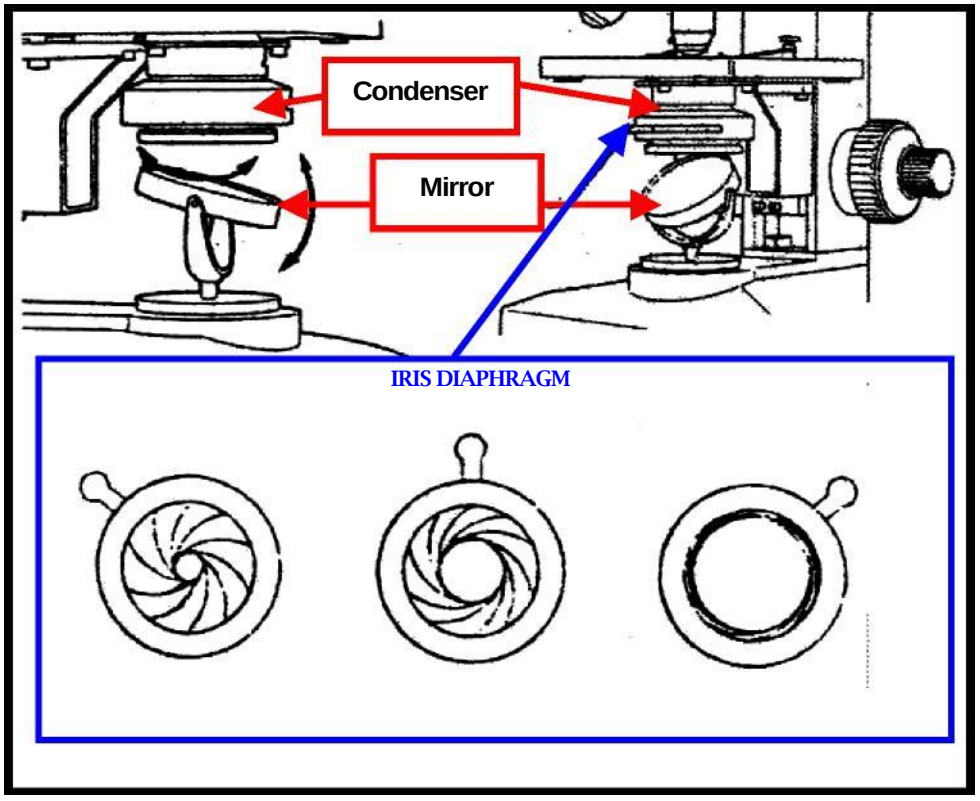
Consist of:

The **mirror** that sends the light to the object.

The **condenser** that focuses the light.

.

The **Iris diaphragm**, which is fixed within the condenser. It reduces or increases the light before it passes through the condenser.



CLOSED *(Obj X10)*

HALF-OPEN *(Obj X40)*

OPEN *(Obj X100)*

THE **ADJUSTMENT SYSTEM**

Consists of:

The **coarse adjustment knob**
This is the largest screw. It is used to adjust approximately.

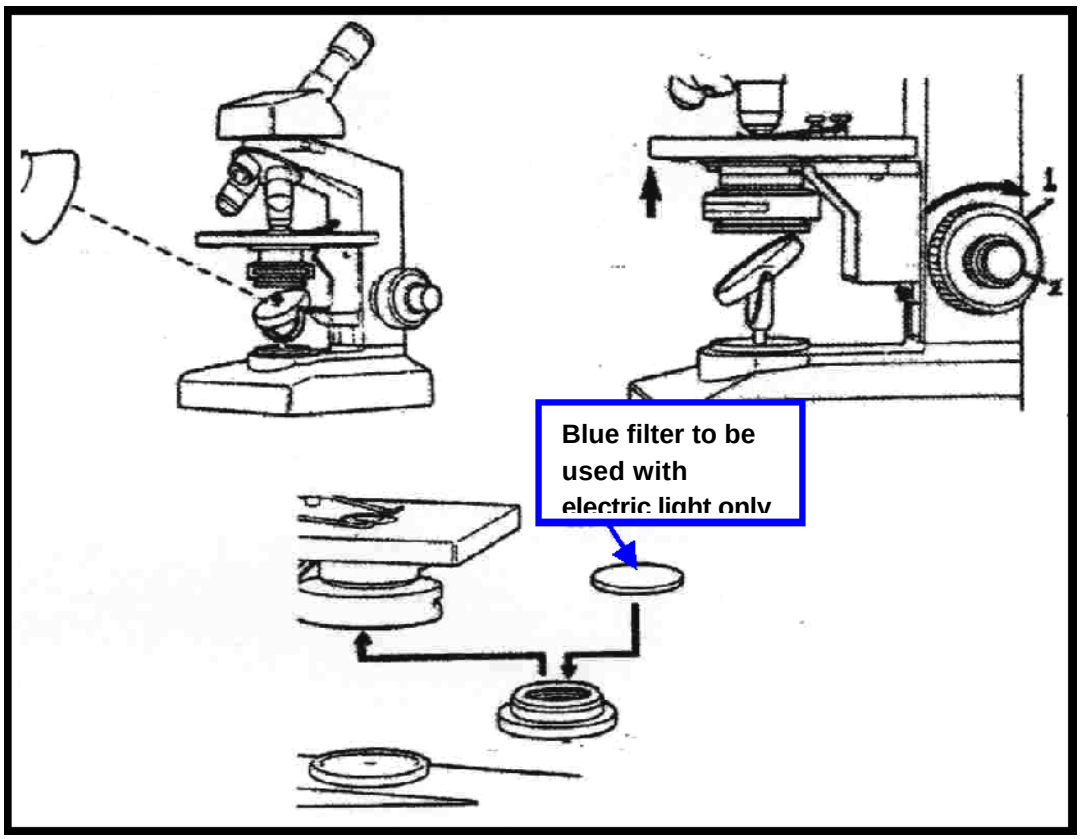
The **fine adjustment knob**
This moves the objective more slowly. It is used to adjust perfectly.

The **condenser knob** This is used to raise the condenser for greater light or to lower it to reduce the light:

Using x 10 objective: condenser down.
Using x 40 objective: condenser half way.
Using x 100 objective: condenser up.

The **iris diaphragm lever**
This is a small lever inside the condenser. It is moved to close or open the diaphragm to reduce or increase the intensity of light

The **two knobs of the mechanical stage.**
One knob moves the slide to the right or left.
The other moves the slide forward or backward.



Setting up the microscope

Place the microscope on a firm bench with a stool to sit comfortably to look in to eyepieces, have space for writing on one side and slides to be examined and immersion oil on the other.
Unless using daylight illumination, sit, and have microscope, facing away from windows.
Most modern microscopes have a built in lamp, some have a detachable lamp that can be replaced by a mirror as shown in the last microscope photo.
Remember to use a blue filter with electric light.
Only use daylight where there is no electricity.

Illumination adjustment

For good illumination it is necessary to line up the light source, with the objectives. This is done by centring both the light source and condenser.

Turn on the light switch and set the light on low with knob or slider.
.
Raise the condenser to its highest position and open the condenser iris diaphragm to two-thirds of its maximum aperture.

Place a tissue over the condenser and adjust the mirror or light source controls to give maximum light. Some microscopes have knobs for centring the light bulb as well as those for centring the light aperture, most will have a Field iris diaphragm for adjusting the amount of light.

Lower the microscope stage using the two course adjustment knobs.

Place a stained slide on the stage and hold it in place with the Slide holder.

Swing the 10x objective into the light path and focus the object on the slide with the course and fine adjustment knobs.

Close both Field and Condenser diaphragms.

Slightly lower the condenser until the edge of the field diaphragm is in focus.
Slightly open the field diaphragm and use the light source adjustments to centre the edge of the field iris in the field of view.
If there are separate knobs for centring the light bulb adjust these to give maximum light.

Once set up, these adjustments do not need to be changed for daily use.

For routine daily use:

Using the 10x objective, move the mechanical stage to find the best area on the slide to examine.

Turn the light to high.

Lower the stage and place a drop of immersion oil on the slide.

Swing the 100x objective in to the light path.
Gently raise the stage until top of the oil touches the objective.
Slightly lower the stage but not enough to break the oil seal.
Carefully raise the stage to focus the slide with the course, and then fine, adjustment knobs.
Close field and condenser diaphragms.
Slightly move the condenser if necessary to focus the edge of the field diaphragm. If necessary re-centre the image of the iris with the light source adjustments and the condenser with its centring screws.
Slightly open the field iris, and adjust condenser iris for best viewing.

The light can be turned down and adjusted to a comfortable viewing level.

The microscope is now ready for use.

Some condensers have a swing out top lens. This is swung in for use with 40x and 100x objectives, and out for lower power objectives.

Notes on examination of Geimsa stained blood films for malarial parasites.

Thick films should first be examined using the 10x and 40x microscope objectives. We do this to select best stained parts of the slide that we will examine closely.
The best part of the thick film should have about 10 White blood cells per High Power Field (HPF). It is sometimes possible to identify other things at this stage, including Microfilaria. We may also see dark brown Malaria pigment in WBCs, this is an important finding even if no parasites are found because it is a clue that malaria parasites have been in the blood.

Raise the objectives above the slide and put a small drop of immersion oil on the selected area before examining with the 100× objective.

ROUTINE MAINTENANCE OF THE MICROSCOPE

The microscope needs daily attention to keep it in good working order to ensure reliable laboratory results.

1- Gently remove the oil from x100 objective with lens paper or soft tissue paper.

2- Clean the eyepieces with lens paper or soft tissue paper.

3- Clean the condenser, the mirror and support stage with a soft tissue or cloth.

4- Cover the microscope with a clean plastic cover in order to protect it from dust.

5- Unless it is kept in air-conditioning or a box with an electric light bulb,

Put it in a sealed microscope box with an opened jar of blue Silica Gel desiccant.
The Silica Gel must be replaced when the colour changes from blue to pink.

Silica Gel can be refreshed by heating it gently in a pan or microwave, the color will turn from pink back to blue and it can be used again.